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FERTILIZER STUDIES WITH RADIOACTIVE SULPHUR. I.

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INTRODUCTION

Sulphur deficiency in Alberta gray wooded soils has been frequently described (12, 13, 15, 20). These reports show that applications of sulphur-containing fertilizers have little direct effect on yield of grass and cereal crops but that yields of legumes are greatly increased. However, cereals and grasses following sulphur-fertilized legumes show marked yield increases. Similar results have been reported in Saskatchewan (2), California (5) and Florida (3).

A sulphur-fertilized crop is often higher in total nitrogen and richer green in colour than the unfertilized crop (1, 3, 5, 13). Cereal crops following sulphur fertilized legumes have also possessed these characteristics (5, 13, 15). The fertilization has usually increased the per cent of sulphur in the crop (1, 3). The nitrogen and sulphur contents of crops grown on the sulphur-deficient soil at Breton Field have been investigated (10, 15) and the results indicate that sulphur-fertilization increased the content of both nitrogen and sulphur. However, the data reported for legume crops were for the hay crop as harvested in the field. This hay consisted of a mixture of alfalfa and altaswede clover plus other miscellaneous plants, mostly weeds. The botanical composition of the plant population varies considerably from plot to plot, with the result that the effect of sulphur fertilization on the composition of alfalfa and altaswede clover produced at Breton Field is not satisfactorily known.

Application of fertilizers containing sulphur to sulphur-deficient soils in other parts of North America has given yield increases similar to those reported for Alberta (1, 2, 3, 4, 5). The percentage yield increases in Alberta for sulphur-fertilized legumes, and for cereal crops following them, are as great as or greater than those reported for other areas, while the amounts of sulphatic fertilizer applied on the average have been lower than those used elsewhere (13). This suggests the possibility that the sulphur deficiency of those Alberta soils which have been investigated is more severe than sulphur deficiencies reported in other parts of North America. More information concerning the nature and effects of sulphur deficiency of wooded soils is desirable because of the very large acreage of these soils in Alberta and because of possible effects of sulphur fertilization on nutritional values of crops produced.

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In order to obtain more specific information concerning the effects of sulphur fertilization on some of the crops grown at Breton Field, this investigation was begun in 1949. The radioactive isotope S^{35} was used as a tracer in order to determine the proportions of soil and fertilizer sulphur in plants from plots dressed with fertilizer.

MATERIALS AND METHODS

Fertilizer

The radioactive sulphur used as a tracer was obtained from the Isotope Branch of the National Research Council, Chalk River, Ontario. The S^{35} was obtained in separate 1 mc. shipments of carrier free $H_2S^*O_4$ solution. Each shipment of $H_2S^*O_4$ was thoroughly mixed with sufficient chemically pure Na_2SO_4 solution to fertilize ten square yards and provide a standard for laboratory use. In the field each of the various fertilizer solutions was applied to 10 one-square-yard plots. Unfertilized check plots were beside each fertilized plot permitting statistical analysis by the "t" test for paired samples (8). About one litre of water was applied to each of the check plots to compensate for the water in the $Na_2S^*O_4$ solution applied to the test plots.

TABLE 1.—DETAILS OF FERTILIZER APPLICATIONS USING $Na_2S^*O_4$

Date of application 1949	Rate of application of Na_2SO_4 lb. per acre	Crop	Previous fertilizer history of soil
June 5	100	Alfalfa and clover	No fertilizer since 1930
June 11	200[1]	Olli barley	No fertilizer since 1930
June 18	100	Alfalfa and clover	NPS since 1930[2]
June 25	100	Olli barley	NPS since 1930[2]

[1] This applied in error. One hundred lb. per acre had been intended.
[2] S at approximately 10 lb. per acre per year.

Soil

The plots at Breton Field (which is 75 miles southwest of Edmonton) are located on Breton loam, a moderately podsolized soil having 4-5 inches of ashy light coloured A_2 horizon. This soil is developed on glacial till. The plots were established by the Soils Department, University of Alberta, in 1930 (13). The 1949 fertilizer treatments outlined above were made in the portion of the field which has been cropped to a 5-year rotation consisting of 3 grain crops and 2 years of mixed legume hay. The plots which had received an NPS fertilizer since 1930 had been given annual applications of approximately 70 pounds per acre of ammonium phosphate 16-20-0, with the exception of the years 1947 and 1949.

Sampling

The first sampling was done July 13-14. All plant material from each square yard in the legume plots was clipped off, the alfalfa and altaswede clover being separated at this time. A six-inch strip, selected at random from one of the 4 central rows of plants and extending across the plots, was harvested from each barley plot. For legumes the nearest edge of the

* The asterisk denotes a preparation containing radioactive atoms.

TABLE 2.—GROWING SEASON MOISTURE AT BRETON

1949 Individual records[1]		Precipitation by periods		
Date		1949		10-year average (incl. 1949)
	inches		inches	inches
April 20	0.35	April 1—30	0.35	0.32
May 17	0.16	May 1—15	0.00	0.49
May 22 and 23	0.30	May 16—31	0.71	1.09
May 27 and 28	0.25	June 1—15	0.00	1.81
June 21 and 22	0.43	June 16—30	0.88	1.66
June 24	0.28	July 1—15	0.62	1.45
June 28	0.17	July 16—31	8.20	2.30
July 5	0.38	Aug. 1—15	0.00	1.15
July 6	0.24	Aug. 16—31	1.30	1.27
July 16 and 17	5.20			
July 19	1.30			
July 22, 23 and 24	1.20			
July 26	0.50			
Aug. 30	1.30			

[1] Precipitation for individual dates is given because most of the moisture falling prior to July 16 was lost by evaporation.

areas harvested for check plots came to within about 6 inches of the plots treated with $Na_2S^*O_4$. For barley the nearest end of the row of plants harvested for check plots came to within about 6 inches of the plots treated with $Na_2S^*O_4$. At the time of the second sampling these separating distances were increased to about one foot. In each case all miscellaneous foreign plants were removed from the samples. Yield data were obtained by taking the air dry weight of the individual samples. At the time of the first sampling there had been practically no rainfall in 1949 and the growth of crops was the poorest in 20 years. (See Table 2).

The second sampling was done on August 24-25 using methods similar to those described above. This sampling followed a 5-week period of unusually heavy rainfall. The barley was just heading and the alfalfa was just beginning to bloom at this time.

The mature barley was harvested September 17 following a very heavy frost.

Sulphur Determination

For analysis approximately 10-gram oven-dry samples were ashed using HNO_3 and $HClO_4$ according to the method of Gieseking *et al.* (7). After destruction of chlorates the residue was taken up in approximately 0.3 N HCl, filtered and diluted prior to precipitation of the sulphate as a fine precipitate of $BaSO_4$. For several samples of relatively high activity the residues from filtration were tested and were found to contain no activity. The precipitates were transferred quantitatively to weighed centrifuge tubes, centrifuged, and washed. After oven-drying the tubes were again weighed.

Method of Counting S^{35}

The dried precipitates from the sulphate determination were suspended in small quantities of ethyl alcohol and transferred to weighed counting dishes (planchets). In the planchet a uniform suspension of precipitate in

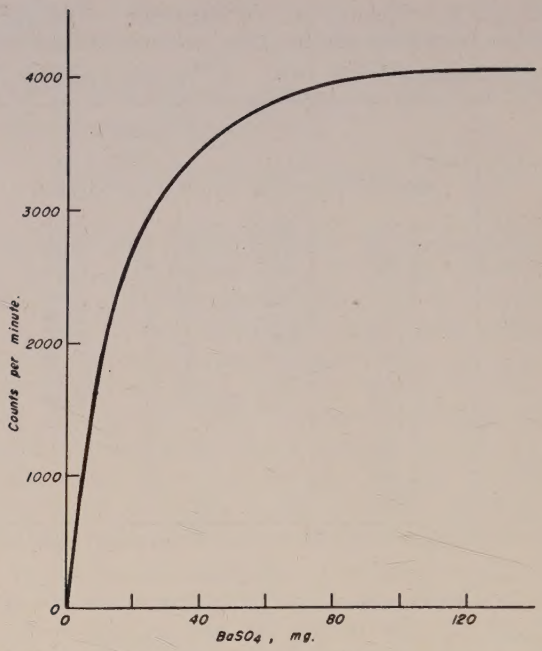

FIGURE 1. Counts on various amounts of BaS^*O_4 of the same specific activity.

alcohol was obtained by "churning" the material with the dropper used for the transfer. For the thick sample procedure described subsequently this produced a satisfactory uniform distribution of precipitate on the bottom of the planchet. The alcohol was then evaporated under a heat lamp and the sample was ready for counting.

An aluminium tray, with a suitable recess to hold the planchets, was used to slide the samples under the window, of the Geiger-Müller tube. The arrangement was such as to ensure reproducible geometry. The samples were slightly less than 1 cm. below the Geiger-Müller tube window which had a thickness of 1.8 mg. per cm^2. The resolving time of the tube was determined by the method of Schweitzer and Whitney (17) to be 5×10^{-6} minutes. The counter was connected to a scale of 64 scaler. Background count, which remained constant at about 30 counts per minutes, was subtracted from sample counts. With the exception of the check plot samples, which had very low activities, the statistical error of counting was reduced to approximately 1 per cent by taking approximately 10,000 counts on each sample.

The performance of the counter was checked at intervals using a uranium standard. The field samples being counted were "sandwiched" between standard samples prepared from the original fertilizer solutions in a manner similar to that described by Spinks and Barber (19).

Self Absorption

S^{35} is a relatively low-energy beta emitter (E_{max} 0.17 Mev.) (17) with the result that self-absorption is an important factor in its measurement. Hendricks *et al.* (9), Spinks and Barber (19), and others have described use

of correction curves to allow for self-absorption. This method was found unsuitable for the work reported here as duplicate determinations of activity frequently differed by more than 5 per cent. Schweitzer and Whitney (17) have pointed out that this is due to the difficulty of preparing $BaSO_4$ samples with constant thickness. They recommend use of samples of such a thickness that radiation from the bottom of the sample is completely absorbed by the sample itself. Samples of this type, referred to hereafter as "thick" samples, were used in this work with the exception of a few cases where the amount of precipitate obtained was insufficient to constitute a thick sample. In these cases it was necessary to use self-absorption correction curves.

Thick samples of S^{35} in the form of $BaSO_4$ are readily prepared. The minimum thickness for thick samples was determined to be approximately 16 mg. per cm.^{2}, a value agreeing well with that of about 15 mg. per cm.^{2} reported by Hendricks *et al.* (9). Using thick samples the maximum difference between duplicate samples was approximately 2 per cent and the majority of duplicates checked considerably more closely than that. Figure 1 is a graphical presentation of data obtained when various amounts of a $Na_2S^*O_4$ solution were precipitated as BaS^*O_4 and counted. As this figure shows, a count made on a thick sample gives the maximum count which can be obtained from a material of that specific activity under the conditions being employed. Specific activity here refers to the number of counts per minute per milligram of BaS^*O_4.

Solutions having known levels of activity were prepared by adding various amounts of carrier-free $H_2S^*O_4$ to solutions containing equal amounts of Na_2SO_4. Figure 2 is a graphical presentation of data obtained by counting thick samples prepared from these solutions. This figure shows that thick sample counts corrected for background are directly proportional to the levels of activity concerned. By using thick samples, therefore, the proportion of sulphur derived from the fertilizer may be determined directly by comparison of counts from plant material samples with counts of standard samples prepared from the original fertilizer solutions (Figure 3).

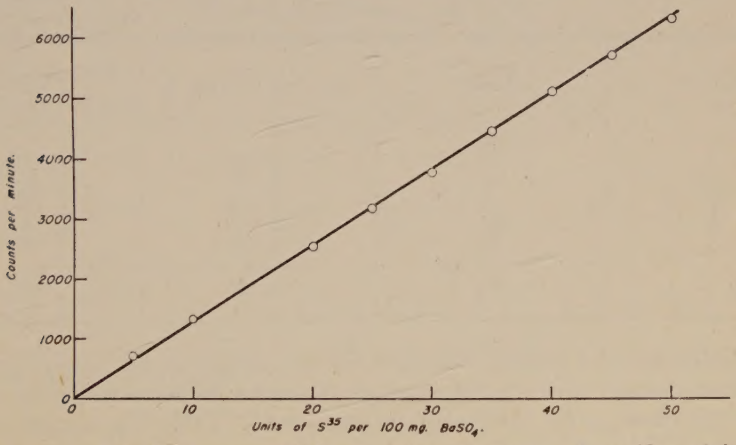

FIGURE 2. Relation between thick sample counting rate and specific activity.

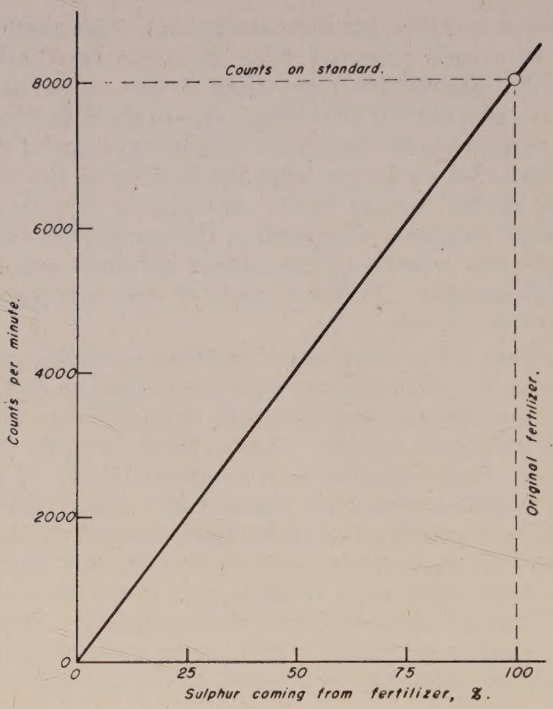

FIGURE 3. Relation between thick sample counting rate and per cent of sulphur coming from fertilizer.

RESULTS

Analyses for total sulphur and for the proportion of sulphur coming from fertilizer were made on only 5 of the 10 field samples obtained from each of the treatments outlined in Table 1. Yields for the 10 plots of each treatment were obtained. These data displayed a degree of variability frequently encountered with such biological materials. A typical set of data for 5 plots is given in Table 3.

TABLE 3.—DATA FOR INDIVIDUAL SAMPLES OF BARLEY FERTILIZED, JUNE 25. SAMPLED, AUGUST 24

Plot No.	Yield	Per cent of total S	Per cent of S from fertilizer
	gm.		
1	33	0.130	43
2	42	0.157	45
3	42	0.175	39
4	44	0.130	41
5	48	0.188	45
Average	42	0.156	42

Tables 4 and 5 present the data for all samplings and analyses. The yield data in each case are the average values for 10 plots while the sulphur values are averages for the 5 samples analysed. Table 6 is calculated from the data of Tables 4 and 5.

TABLE 4.—YIELD AND SULPHUR DATA FOR LEGUMES

	Yield, gm. per square yard		Total S, per cent		Per cent of S in plant which came from fertilizer	
	Check	Fertilized with $Na_2S^*O_4$	Check	Fertilized with $Na_2S^*O_4$	Check	Fertilized with $Na_2S^*O_4$
Alfalfa—						
First sampling:						
No fertilizer since 1930	15.2	26.2**	0.10	0.18	2	54
NPS since 1930	24.0	28.4	0.10	0.12	1	31
Second sampling:						
No fertilizer since 1930	26.0	110.1**	0.08	0.12	20	80
NPS since 1930	50.9	88.5**	0.11	0.19	1	63
Altaswede clover—						
First sampling:						
No fertilizer since 1930	10.7	15.2	0.13	0.23	9	72
NPS since 1930	17.3	36.2**	0.09	0.10	1	38
Second sampling:						
No fertilizer since 1930	20.2	60.9**	0.08	0.15	1	73
NPS since 1930	53.9	68.4**	0.09	0.17	1	60

** Highly significant yield increase.

TABLE 5.—YIELD AND SULPHUR DATA FOR OLLI BARLEY

	Yield, gm. per square yard		Total S, per cent		Per cent of S in plant which came from fertilizer	
	Check	Fertilized with $Na_2S^*O_4$	Check	Fertilized with $Na_2S^*O_4$	Check	Fertilized with $Na_2S^*O_4$
First sampling:						
No fertilizer since 1930	59.6	51.6	0.14	0.25	1	56
NPS since 1930	66.0	63.0	0.19	0.20	1	42
Second sampling:						
No fertilizer since 1930	125.4	153.0	0.13	0.16	31	64
NPS since 1930	244.2	261.0	0.12	0.16	0	42
Mature crop—						
Straw:						
No fertilizer since 1930	239.0	301.0**	0.11	0.15	24	59
NPS since 1930	376.0	404.0	0.09	0.11	0	46
Grain:						
No fertilizer since 1930	53.0	44.0	0.12	0.14	22	61
NPS since 1930	75.0	76.0	0.14	0.15	0	44

** Highly significant yield increase.

TABLE 6.—AMOUNT OF SOIL SULPHUR REMOVED BY CROPS

	Soil sulphur in mg. per square yard					
	Clover		Alfalfa		Total for hay	
	Check	Fertilized	Check	Fertilized	Check	Fertilized
No fertilizer since 1930—						
First sampling	13.9	9.8	15.2	22.1		
Second sampling	16.2	24.7	20.8	26.4		
					66.1	83.0
NPS since 1930—						
First sampling	15.6	22.4	16.7[1]	23.5		
Second sampling	48.5	46.5	56.0	62.2		
					136.8	154.6

[1] Adjusted for the fertilizer sulphur taken up by these "checks'

DISCUSSION

Effect of Weather Conditions

The results are for crops grown in a single season. The season was an unusual one with unprecedented drought preceding the first sampling and an excessively moist period preceding the second sampling. It is possible, therefore, that results secured in more nearly normal seasons would differ appreciably from those now reported.

Validity of the Method

The validity of the use of radioactive isotopes as tracers is now rather generally accepted. However, the preferential uptake of radioactive isotopes because of a disturbance of the isotopic equilibrium has been described by Overstreet and Broyer (14). Although this may be a factor of some consequence in hydroponic work where plants with a high content of certain elements come in contact with supplies of tracer-tagged nutrients, it is unlikely that results reported here have been affected in this way. At the time of fertilization the plants at Breton were very small, and low in sulphur content. These conditions practically eliminate the possibility of any appreciable effect such as the one reported by Overstreet and Broyer.

Effect of Radiation Damage

In spite of the adverse weather the results illustrate the yield effects of sulphur fertilization. Legume yields, obviously improved as determined by visual inspection of the plots, were increased by as much as 400 per cent. Fertilizer uptake by one set of barley "check" plots renders unusable the associated yield data. However, close observations in the field did not reveal any visible effect on barley yield as a result of the fertilization with $Na_2S^*O_4$. The lower yields of barley on the sulphur-fertilized plots at the time of the first sampling suggest the possibility of injurious effects of the radioactive isotope S^{35}.

Russell and Adams (16) have discussed the possible injurious effects of P^{32} when used in fertilizer as a tracer. They suggest that the maximum non-injurious level of P^{32} in their experiments was 10 μc per litre of nutrient

solution used. In the work reported here the rate of fertilization provided approximately 0.5 μc per kilogram of soil for the top $6\frac{2}{3}$ inches. Initially the S^{35} was confined to a soil layer considerably less than $6\frac{2}{3}$ inches deep. The activity in this layer was therefore considerably higher than calculated above. Due to the differences in the half-lives and the energies of S^{35} and P^{32} it is difficult to compare the effects of the two radiations on plants. It would be desirable to investigate this in the case of S^{35}.

Results for Barley

Since, in one case, the so-called "check" plots of barley contained large amounts of fertilizer sulphur, conclusions concerning the effect of the fertilization on yield and sulphur content of this barley are not justified.

The barley "check" plots showing the high proportion of fertilizer sulphur were down slope from the fertilized plots with which they were paired. The uptake of fertilizer sulphur by these "check" plots is attributed largely to a lateral down-slope movement of fertilizer sulphur on and in the soil. Other barley check plots which were up slope from the treated plots with which they were paired did not show any fertilizer uptake under similar sampling conditions. The results emphasize the need for great care in sampling small plots in fertilizer experiments.

The data show that approximately one-half of the sulphur in the fertilized barley came from the fertilizer. The total sulphur content of the barley grain and straw compare rather closely with those quoted by Collings (4).

Results for Legumes

The total sulphur content of the unfertilized legumes is low compared to some reported figures (1, 3, 4). The fertilized legumes had a higher total sulphur content in every case, but Bledsoe and Blaser (3), and Alway (1) give figures showing considerably higher sulphur contents for sulphur-fertilized legumes. Rates of sulphur application at Breton Field since 1930 (13) and in the 1949 tracer work have been generally lower than those used in most experiments on sulphur deficient soils elsewhere. In the work reported here the proportion of fertilizer sulphur was higher in plants grown on previously unfertilized plots in every case. However, the fact that plants grown on previously fertilized plots contained approximately three-quarters as high a proportion of fertilizer sulphur, suggests that fertilization has not resulted in an accumulation of a large supply of readily available sulphur in the soil. Calculations based on data reported here and yield data previously reported for the Breton Field suggest that fertilizer applications made since 1930 have supplied only slightly, if any, more sulphur than that which has been removed in crops.

Although there is no statistical significance the legume data in Table 6 suggest that the fertilization may be causing an increased uptake of soil sulphur. Nellar (11), Collings (4) and others have mentioned the possibility of sulphur fertilization stimulating the symbiotic nitrogen-fixing bacteria. An increase in the supply of nitrogen available to the legumes as a result of Rhizobia stimulation might cause increased removal of soil sulphur by the crop. It would be desirable to determine whether the sulphur fertilization is causing a definite increase in uptake of soil sulphur.

SUMMARY

During the spring of 1949 applications of Na_2SO_4, containing the radioactive isotope S^{35} as a tracer, were made to some plots on the sulphur-deficient soil at Breton Field. Applications were made to plots not fertilized since 1930 and to plots which had received applications of an NPS fertilizer ever since 1930. The crops concerned were an established stand of a legume hay mixture (alfalfa and red clover) and barley seeded as a companion crop for the hay mixture. Large increases in the per cent of sulphur were found in all samplings of the legume crop. Legume yield increases, very conspicuous in the field, were as high as 400 per cent. Since plants from one set of barley "check" plots contained significant amounts of fertilizer sulphur effects of the fertilization on the sulphur content and yield of barley are uncertain. However, about one-half of the sulphur in fertilized barley came from the fertilizer. There was no visible increase in barley growth as a result of the fertilization. The amount of sulphur in the fertilized plants which came from the fertilizer varied from 31 per cent to 80 per cent. In most cases the legumes had higher proportions of fertilizer sulphur than the barley. Plants growing on the previously unfertilized plots showed greatest yield increases and also showed the greatest uptake of fertilizer sulphur.

ACKNOWLEDGMENT

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At the invitation of J. W. T. Spinks, one of the authors visited the Chemistry Department, University of Saskatchewan, in order to become familiar with the use and handling of radioactive isotopes as tracers. This courtesy has been greatly appreciated; it has facilitated the present work considerably.

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SAWDUST AS A MULCH FOR STRAWBERRIES

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Strawberries are the most important of the small fruits grown in British Columbia. They are particularly adapted to the coastal areas of southern British Columbia.

The lumber industry is very large and thriving in this area and the cut of hemlock is increasing each year, so that large quantities of hemlock sawdust are available in progressively increasing amounts.

The object of this experiment was to ascertain whether this hemlock sawdust could be used as a mulch for strawberries with beneficial effects and so prove a boon to both industries instead of a waste product.

Physical and chemical effects of mulches such as straw, hay and manure have been extensively investigated but the effects of sawdust on the soil have come under consideration only in recent years. Midgely (7) points out that woody materials when added to the soil act like other carbonaceous materials. Anderson (1) and Thom (13) have reported that the addition of straws and cellulose to the soil depress the available nitrogen. Turk and Partridge (15) state that nitrification was slower under mulched than unmulched soils. However, they agree with Anderson (1) that soil processes are not inhibited by the addition of a carbohydrate and that actually soil conditions are improved for the development of micro-organisms which utilize the available nitrogen for their growth, the nitrogen usually becoming a limiting factor.

Shatuk *et al.* (12) found that sawdust applied as a mulch to blueberries increased the soil moisture but the increase was not so great as with straw. Turk (14) considers that the effect of sawdust is largely physical. He reports better water holding capacity, decreased soil losses due to erosion, prevention of a hard crust on the soil surface and that capillary movements of water may be limited by the addition of sawdust to the soil.

Most of the humus in soil is derived from, or is lignin in a modified form (5). The end product in the wood decay process is humus and as a result of this conversion considerable amounts of nitrogen, phosphorous and potash may be deposited (2). Turk (14) states that, on an average, sawdust contains 4 pounds of nitrogen, 2 pounds of phosphorus and 4 pounds of potash per ton of dry sawdust; this is about one-third the amounts of nutrients contained in the same amounts of wheat straw. Analyses of sawdust and wood shavings used as bedding material show the following: nitrogen 0.4 per cent or 8 pounds per ton; phosphorous 0.3 per cent or 6 pounds per ton; potash 0.7 per cent or 14 pounds per ton (17). The amounts of nutrients, however, will vary with the type of wood from which the sawdust is derived.

Shatuk *et al.* (12) report that the soil pH was significantly higher under sawdust-mulched blueberry plots than under straw-mulched or clean cultivated blueberry plots. Midgely (7) states that the suggestion that sawdust produces excessive soil acidity has no basis. He states further that soft wood sawdust contains less basic materials than hardwood saw-

dust; yet after a complete decay, most of the organic acids are used by the micro-organisms or lost into the air, leaving an alkali or neutral residue in the soil. All plant ashes are alkaline in reaction. Midgely concludes that, while some plant residues contain certain tannins, these are destroyed by the soil organisms before they remain long in the soil.

Shatuk *et al.* (12) found that blueberry yields were significantly higher in plots mulched with sawdust than in plots mulched with manure or in clean cultivation.

MATERIALS AND METHODS

The experiment was carried out on the farm of the University of British Columbia. The soil is one of the typical soil types of coastal British Columbia. It can be described briefly as an upland glaciated sandy loam with a reaction of about pH 6.0. The organic matter content is low and, due to the leaching effect of the heavy winter rains in this area, available nutrients in general are low.

An area approximately one acre in size was selected and divided into 72 plots. Each plot was planted with certified British Sovereign plants and consisted of 5 rows of 10 plants each, or 50 plants spaced 18 inches within the row and 3 feet between rows. Guard rows were placed between all plots. The British Sovereign variety of strawberry is a local product and is used by growers almost exclusively in the lower Fraser Valley and on Vancouver Island, the two largest strawberry-producing areas in British Columbia.

Twenty-four randomized plots received an application of barnyard manure at the rate of ten tons per acre. Another 24 plots were covered with a layer of freshly cut hemlock sawdust to a depth of 2 inches, while the remaining 24 plots were left for clean cultivation. The manure was disked under before planting, but the plants were planted in the sawdust plots directly by pushing back the sawdust and inserting the planting spade deep enough to penetrate the soil to the required depth for the roots. The whole area received an application of 8-10-5 fertilizer, broadcast at the rate of 1000 pounds per acre. The planting took place on May 30, 1947. Cultivation was practised in the manured and clean cultivated plots while the few weeds which penetrated the sawdust were removed with a Dutch hoe.

On August 15, 1947, leaf samples were taken from each plot for analyses and then a further application of 8-10-5 fertilizer was made at the rate of 500 pounds per acre.

On September 4 a count was made of the number of runners on each plant.

In January, 1948, the sawdust plots received a further 2-inch application of sawdust. The original application had worked down to a considerable extent into the light soil. The ground was frozen at the time, which greatly facilitated spreading the sawdust. In March, 1948, the manure application was repeated and a 1000 pounds per acre of 8-10-5 fertilizer broadcast over the whole area. The treatments were continued as in the previous year except that the crop was harvested and all runners were kept removed from the plants.

The yield data were recorded separately from each plot and analysed statistically by the method of analysis of variance. Samples of fruit from each plot were analysed for ash, carbohydrates, acidity and vitamin C.

The whole area was again fertilized with 8-10-5 fertilizer in August, 1948, at the rate of 300 pounds per acre.

The winter of 1948-49 was very severe for the district, the temperature dropping to 10° F. Very little snow was on the ground, with the result that considerable heaving of strawberry plants was a common occurrence in the district.

In 1949 no further addition of sawdust was made; otherwise the treatments were continued the same as in previous years. The fruit was harvested and data were recorded for yield, sugars, ash, acidity and vitamin C content, together with nitrogen, phosphorous, potash, calcium and magnesium.

Soil samples were taken just prior to setting out the plants in 1947 and again in August, 1948, and 1949. They were analysed for organic matter, nitrogen, phosphorous, potash, calcium and magnesium.

The methods of analyses used were as stated below:

Soil.—Numerous difficulties beset the accurate determination of organic matter. However, as a relative index and for comparative purposes, the wet method of oxidation where the soil is digested with chromic and sulphuric acids was decided upon as being a reasonable method and because of its convenience in determining large numbers of samples. Soil samples were taken to a depth of six inches after the surface sawdust or trashy material, in the case of the manured plots, had been removed. The samples were air-dried and passed through a sieve with round holes of 0.5 mm. in diameter. Ten gram samples of the sifted soil were used for each determination which was made by the rapid titration method of Schollenberger (10, 11). All samples were run in triplicate. Nitrogen was determined by the Kjeldahl method A.O.A.C. (3). A hydrochloric acid extract was made for the determination of calcium, magnesium, potassium and phosphorous as outlined by Wright (16). Potassium was determined by the method of Peech *et al.* (8); calcium and magnesium according to Blasdale (4); phosphorous by the blue colorimetric method of Deneges as modified by McCance and Shipp (6). Soil reaction was determined with a Beckman pH meter using a glass electrode.

Fruit and Leaves.—Ashing was done at 600° C. in an electrical muffle furnace. The dry-weight of leaves was determined by drying in an electric oven at 70° C. and that of the fruit at 60° C. Carbohydrates were determined by the method of Lane and Enynon (3) and vitamin C by the Indophenol-Xylene extraction method of Robinson and Stotz, using a photoelectric colorimeter (9).

RESULTS

Soil

The results of the effect of the treatments as determined by the analyses of the soil samples taken initially when the plants were set out in May, 1947, and in August, 1948, and in August, 1949, are shown in Table 1.

This table shows that the results of the pH determinations are in accord with those of Shatuk *et al.* (12). The pH was significantly higher under the sawdust mulch, in the final analysis, than under the other treatments.

The first year after the treatments only the manured plots showed an increase in organic matter. By the second year, however, the organic matter in the sawdust-treated plots equalled that in the manured plots and finally surpassed them.

The mineral content (ash) of the soil progressively increased as a result of the fertilizer treatments with the exception of that in the sawdust plots. As will be discussed later, the larger plants and crop produced could be responsible for this lack of increase in minerals in these plots.

Nitrogen as nitrates in the sawdust plots did not increase as a result of the treatments the first year. This is in accord with other workers (5, 15, 2, 14). The final values determined for total nitrogen showed a depressing effect of the sawdust but again, as will be seen later, this did not have any deleterious effect on the crop. Either the larger plants used more nitrogen from these plots, or there was ample nitrogen for the crop even with the reduced amount available.

TABLE 1.—THE EFFECTS OF TREATMENTS ON SOIL

Factor	Treatment									L.S.D., 5 per cent
	Manure			Clean cultivation			Sawdust			
	May, 1947	Aug., 1948	Aug., 1949	May, 1947	Aug., 1948	Aug., 1949	May, 1947	Aug., 1948	Aug., 1949	
pH	5.68	5.70	5.70	5.66	5.77	5.70	5.70	5.90	6.02	0.086
O.M., per cent	1.50	2.17	5.20	1.70	1.90	1.75	1.70	1.90	5.80	0.288
Ash, per cent	0.340	0.410	0.450	0.340	0.370	0.450	0.350	0.370	0.384	0.058
NO 3*	28.00	45.50	—	29.00	42.50	—	29.00	29.00	—	3.33
Total N, per cent	—	—	0.139	—	—	0.09	—	—	0.017	0.008
P.*	10.00	33.20	—	5.50	22.10	—	6.00	20.80	—	1.580
Total P., per cent	—	—	0.128	—	—	0.120	—	—	0.082	0.009
K.*	48.00	98.40	48.40	48.00	74.00	47.20	48.00	31.42	83.40	8.82
Ca.*	—	—	256.00	—	—	246.00	—	—	236.00	18.07
Mg.*	—	—	35.00	—	—	30.00	—	—	47.00	3.90
B. p.p.m.	—	—	2.81	—	—	3.10	—	—	6.33	0.326

* Pounds per acre (2 million).

Phosphorous was depressed under the sawdust; potash was less for the first year but eventually it increased markedly.

Calcium appeared to be depressed under sawdust in contrast to the manured plots, whereas magnesium was increased.

Boron increased under the sawdust treatments.

During a period in July, after a prolonged dry spell, soil samples were taken under the various plots to ascertain the effect of the mulch on the water-holding capacity of the soil. The results shown in Table 2 indicate the amount of moisture present in the soil at the time of sampling.

TABLE 2.—THE EFFECT OF SAWDUST MULCH ON WATER HOLDING CAPACITY OF THE SOIL

Treatment			S.D. 5 per cent
Manure	Clean cultivation	Sawdust	
%	%	%	
7.80	8.10	11.7	1.02

It can be seen that the sawdust mulch was effective in increasing the water-holding capacity of the soil.

Plants

It was thought that runner production should be some index of vigour in the plant. Consequently, from the time of planting, runners were counted until September. Owing to the late planting, runner production had by no means ceased at this time, but, from this period on, they were removed without counting. Table 3 shows the counts obtained during the period mentioned.

It should be stated that in the reasonably sheltered experimental area used the runners rooted readily in the compacted sawdust. However, it is conceivable that in very windy areas a pegging-down of runners might be desirable. In this particular experiment, after the initial counting of runners mentioned above, the Hill system of growing was adhered to. The Hill system is used almost exclusively in growing the British Sovereign variety in British Columbia.

TABLE 3.—THE EFFECT OF TREATMENTS ON RUNNER PRODUCTION

	Treatment		
	Manure	Clean cultivation	Sawdust
Average number of runners per plant	7.98 ± 1.03	9.52 ± 1.03	12.6 ± 1.03

TABLE 4.—THE EFFECT OF TREATMENTS ON LEAF COMPOSITION (1947)

Factor	Treatment			L.S.D., 5 per cent
	Manure	Clean cultivation	Sawdust	
	%	%	%	%
Dry weight	28.00	28.85	27.92	2.24
Carbohydrate, d.w.	30.39	28.09	30.85	2.89
Nitrogen, d.w.	2.317	2.145	2.2975	0.171
Ash, f.w.	0.647	0.646	0.634	0.045
Phosphorous, d.w.	0.313	0.330	0.290	0.021
Potash, d.w.	1.50	0.96	1.35	0.112

It will be observed that the plants in the sawdust-treated plots produced the most runners.

An analysis of leaves taken from the plants in August, 1947, is shown in Table 4.

Carbohydrates, nitrogen and potash were lowest under clean cultivation, while phosphorous was so in the sawdust-treated plots.

Fruit

The effect of the treatments on the mineral composition of the fruit from the 1949 crop is shown in Table 5.

TABLE 5.—THE EFFECT OF TREATMENTS ON THE COMPOSITION OF THE FRUIT (1949 CROP)

Factor	Treatment			L.S.D., 5 per cent
	Manure	Clean cultivation	Sawdust	
	%	%	%	%
Dry weight	14.89	13.96	14.34	1.04
Ash, f.w.	0.544	0.483	0.505	0.035
Nitrogen, f.w.	0.162	0.148	0.150	0.018
Phosphorous, f.w.	0.032	0.036	0.031	0.003
Potash, f.w.	0.785	0.670	0.765	0.068
Calcium, f.w.	0.048	0.047	0.039	0.006
Magnesium, f.w.	0.016	0.015	0.021	0.004

TABLE 6.—EFFECT OF TREATMENTS ON YIELD AND COMPOSITION OF FRUIT FOR EACH CROP YEAR 1948 AND 1949

Factor	Year	Treatment			L.S.D., 5 per cent
		Manure	Clean cultivation	Sawdust	
Yield, lb. per plot	1948	30.03	27.38	27.07	4.90
	1949	58.00	52.00	120.00	14.60
	Av.	44.00	39.69	73.50	6.22
Ash, f.w., per cent	1948	0.5520	0.5435	0.5932	0.0394
	1949	0.5440	0.4821	0.5048	0.0408
	Av.	0.5480	0.5128	0.5490	0.0320
Sugars, f.w., per cent	1948	9.47	9.63	9.16	0.6094
	1949	9.45	8.80	8.27	0.5188
	Av.	9.46	9.21	8.74	0.450
pH	1948	3.28	3.29	3.29	0.020
	1949	3.28	3.28	3.33	0.035
	Av.	3.28	3.29	3.31	0.019
Vitamin C, mgm./100 gm., f.w.	1948	95.50	94.16	92.11	5.48
	1949	82.00	90.00	98.00	6.30
	Av.	88.75	92.08	95.05	4.32

From the foregoing results it would appear that the mineral (ash) content of the fruit was the highest in that from the manured plots, phosphorous was highest in fruit from the clean cultivated plots, potash was lowest in fruit from the clean cultivated plots, calcium was lowest in fruit from the sawdust-treated plots and magnesium was highest in fruit from the sawdust-treated plots.

Table 6 shows the data which were obtained on yield of fruit and from the chemical analyses of the same, for each crop year 1948 and 1949.

Yield

In 1948 the yield of fruit from the manured plots tended to be the highest, although the results were not significant at the 5 per cent level. In 1949, however, the sawdust plots greatly outyielded both the manured and clean cultivated plots. The combined results of the two years showed that the sawdust plots outyielded the manured and clean cultivated plots.

Ash.—The sawdust plots produced berries with the highest mineral content in 1948. In 1949 a reversal took place and the manured plots produced berries with the highest mineral content. In the combined results the manured and sawdust-treated plots both produced berries of a higher mineral content than did the clean cultivated plots.

Sugars.—The 1948 results show a trend towards the highest sugar content in the berries from the clean cultivated plots. In 1949 the berries from the manured plots showed the highest sugar content. The combined results show that the sawdust had depressed the sugar content of the fruit.

Acidity.—The acidity of the fruit from the sawdust plots showed a tendency to decrease in 1948 and a definite decrease was apparent in 1949. The combined results for both years show a significant decrease in acidity as a result of the sawdust treatment.

Vitamin C.—In 1949 the fruit from the sawdust plots showed the highest vitamin C content and that from the manured plots the least. The combined results show that the sawdust plots produced berries of higher vitamin C content than did the manured plots.

DISCUSSION

It was obvious throughout the experiment that no harmful effect was derived from the application of the sawdust. In fact, everything pointed towards its beneficial nature. There was evidence that nitrification was slower under the sawdust than in the clean cultivated or manured plots, but the soil processes were not inhibited by its addition. This is in agreement with the workers previously mentioned (1, 15). The physical improvement of the soil was noteworthy and, after the first year, breakdown of cellulose and hemicellulose was apparent by the large increase in organic matter in the soil shown in the 1949 analyses, Table 1. The decrease in acidity of the soil under the sawdust is in accord with Shatuk *et al.* (12).

The effect of the sawdust mulch in preventing heaving of strawberry plants was very pronounced as evidenced by the effect of the hard winter of 1947-48. This could account in part but not altogether for the higher yields from the sawdust plots in 1949.

The depressing effect on nitrogen and phosphorous in the soil by the sawdust was not reflected in the fruit when compared with that from the clean cultivated plots. In view of the larger crop from the sawdust plots and hence the greater drain on the soil for nutrients it could either be interpreted that the sawdust made more nutrients available to the plant or that the greater crop reduced the soil nutrient content in a degree proportionate to the increased amount used by the larger crop. On the other hand, all plots were well and adequately fertilized so that the minerals and nitrogen were not limiting factors in this experiment. The soil under the sawdust plots did have a lower calcium content and a higher magnesium content than that of the other plots. This was reflected in the fruit, whereas there seemed to be no similar relationship between fruit and soil with the other minerals and nitrogen. Possibly calcium and magnesium were nearer to limiting values in the soil than the other constituents determined.

The depressing effect of sawdust on sugars in the fruit seems to be a factor to be considered in sawdust mulching. However, associated with this lowering of sugar was the increased vitamin C content and tendency towards a higher pH of the berry. The inverse relationship here shown between sugars and vitamin C is difficult to explain, unless the higher pH in some way affected the factors concerned in the more effective metabolism of the ascorbic acid and proportionally more of the sugars were used in the process.

SUMMARY

Sawdust mulching did not appear to be harmful to strawberries; in fact, taking all factors into consideration, it was beneficial. Besides improving the physical condition of the soil it prevented heaving of the plants during a severe winter and its after-effects.

While sawdust did reduce the nitrogen content of the soil, this condition was not reflected in the fruit. Nitrogen, however, under the conditions of this experiment was adequately supplied.

Yields of fruit were increased markedly by the use of the sawdust mulch the second year after its application, and while there was reduction in the sugar content of the fruit there was an increase in vitamin C content.

Hemlock sawdust would appear to have possibilities of becoming very beneficial to the strawberry industry and to agriculture in general as a soil amendment and mulching material. No harmful effect on the soil by its use was noted. This should create an outlet for increasing quantities of hemlock sawdust which might otherwise be considered a waste product, thereby benefiting the lumber industry as well as agriculture.

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FACTORS IN RELATION TO TUBER QUALITY IN POTATOES

II. PRELIMINARY TRIALS ON BITTERNESS IN NETTED GEM POTATOES[1]

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In recent years, increased irrigation facilities and land valuations generally in parts of Western Canada have caused agricultural producers to increase intensive cropping. Crops so produced include sugar beets, potatoes, and vegetables for canning and freezing. Periodically, according to experienced potato-growers in Alberta, a proportion of a season's produce of the Netted Gem variety will have distinctly acrid flavour when the tubers have been boiled. This may vary, even on one farm, with the season, with the method of cooking and with the elapsed storage-time. Occasionally, other varieties in Alberta and elsewhere in Canada and the United States are reported as having this acrid flavour.

For some time prior to the present century, in certain seasons bitterness in potato tubers has been observed to be present in more than the usual degree, and discussions, augmented with some early experimental work and analytical technique, were published in several European countries. Eventually, general agreement was reached as to the cause of the bitterness; and it has been identified as solanine, a complex that resembles an alkaloid, and probably is one. It is particularly noticeable in potato tops and sprouts, and the bitter tang of its taste probably accounts for the distaste for potato sprouts and tops often observed in domestic foraging animals.

On a few occasions, serious illness and death have been attributed to high solanine content in potato tubers, but such occurrences are very rare. Of more frequent commercial importance is the fact that complaints from consumers of Netted Gem potatoes have led to sales losses and could readily result in serious acreage reduction and consumer discrimination unless remedial or preventive measures can be taken. So far, experimental work has resulted in many statements that are quite contradictory regarding causal factors. These statements are certainly not very helpful to producers, other than to point to the complex nature of the problem.

LITERATURE REVIEW

According to Meyer and Anderson (16), alkaloids are generally believed to be by-products of the nitrogen metabolism of the species in which they are found. Solanaceous species are notably productive of poisonous alkaloids, and according to Henry (11) and Rooke *et al.* (22), a compl ex glucoside found in *Solanum tuberosum* is solanine. This is also referred to as a "glycosidal alkaloid", and various suggestions have been made as to the empirical formula of the compound. These have been reviewed by Conner (5) in 1937, who points out the lack of uniformity in estimates of the structure of solanine, as well as in the suggested causes of high alkaloid content of potato tubers. Hansen (19) and Henry (11) point out that

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solanine is unaffected by alkalis, but can be hydrolyzed, with warm acid treatment, into the base solanidine and one molecule each of rhamnose, galactose and dextrose. The empirical formula of solanine is now regarded by Rooke and his co-workers (22) as $C_{45}H_{73}O_{15}N$, and that of solanidine to be $C_{27}H_{43}ON$. Hardenburg (10), on the other hand, gives the formula as $C_{52}H_{83}O_{13}N$, and describes the material as "an amorphous alkaloid". According to Clem *et al.* (4), solanine has a steroid structure and contains secondary alcohol and tertiary nitrogen groups.

The properties of solanine have been determined by several workers (1, 5, 6, 11, 22). It is insoluble in water, or practically so; very slightly soluble in cold alcohol; readily soluble in hot alcohol; insoluble in ether, chloroform, benzene and petroleum ether. Amyl and ethyl alcohols are used to extract solanine from its alkaline plant juice solutions. It is very weakly basic and does not reduce Fehling's solution, but will reduce certain other copper compounds and sodium thiosulphate.

According to Davis and Stadtler (6), solanine is bitter and pungent, leaving in the pharynx a persistent acrid sensation. It is poisonous, one-quarter grain being strongly nauseating to a man; odourless when dry, but when moist it exhales an odour recalling that of cooking potatoes. Henry (11) describes the solanines as "protoplasmic poisons and potent hemolytics", and points out that symptoms of solanine poisoning from potatoes are headache, nausea, emesis and gastritis.

Morgenstern (17, 18), one of the early workers with solanine, determined that 18 table potato varieties averaged 0.0125 per cent solanine, while seven stock varieties averaged 0.0058 per cent. Yellow potatoes contained less than red or blue varieties. Other workers (12, 23) reported varietal differences, and Lampitt (12) found that solanidine, a basic component of solanine, occurred in free form in sprouts of some varieties but not in others. Gortner and Gortner (8) state that potatoes normally have about 24 parts per million (0.0024 per cent) of solanine while Bomer and Mattis (3) give a normal range of 0.002 per cent to 0.0089 per cent solanine. The latter workers suggest that tubers with more than 0.020 per cent solanine were beyond the upper safety limit for food purposes. Lepper in a 1939 report (13) showed that even within a variety or a single lot of potatoes, the solanine content can vary widely, and variation within the same lot of tubers sampled at different storage intervals was reported in 1939 by Naumov (19).

While Morgenstern (17) concludes that applications of nitrogen and phosphorus fertilizers increase solanine in tubers, and lighter soils also were associated with increases in solanine, other workers (3, 14, 20) have reported no effect of fertilizers and soil types upon the content of the alkaloid. One of the few points of general agreement among reporters was that immature tubers were likely to have a higher solanine content than when they had matured; also that smaller tubers contained more solanine than larger ones. In Armenia, summer cultivation of potatoes grown in hot, valley climates is recommended to reduce solanine content (2).

Bitterness in tubers has long been associated with "greened" tubers, and the latter is due to the development of chlorophyll in the tuber skin. Thus, because "sunburned" potatoes are often bitter, it has been popularly

thought that the bitterness was positively correlated with the amount of "sunburn" or greening. Conner (5), working in Illinois, found that light rays from the blue end of the spectrum were most effective in encouraging solanine formation whereas the yellow-red end of the spectrum was most efficient for chlorophyll and subsequent glucose formation, but did not increase the solanine significantly.

Wolf and Duggar (23), and other workers (3, 12, 14, 16, 18) have noted an association of high solanine and greened tubers, and also an extremely high content in irradiated potato sprouts. They conclude that solanine is found in greatest concentration in those organs and tissues where metabolic activity is greatest, such as tops, sprouts, near eyes on the tuber and in the outer cortex of tubers generally.

Mayfield *et al.* (15), studying the effect on the quality of Montana-grown Netted Gem and Bliss Triumph potatoes of winter storage conditions, noted increases in solanine content from 0.008 per cent in August to 0.057 per cent in May. The latter amount would seem to be well into the danger zone for poisoning, as noted by Bomer and Mattis (3). Mayfield recorded that Netted Gem potatoes were much more likely to develop bitterness, as their storage-time increased, than were tubers of Bliss Triumph, and that the bitterness in the former variety seemed to be associated with a distinct greenish-yellow flesh colour. They state also that warm, dry storage (home basement, with weak light entering) caused no increase in bitterness in either variety, but that the Netted Gem tubers developed their highly acrid flavour in a cool, moist storage, with light excluded. Analyses for solanine content on the two varieties showed that each increased at about the same rate, even though the acrid flavour was found only in the Netted Gem.

The opinions expressed by the majority of workers whose papers have been reviewed, may be summarized as follows:

(1) The compound responsible for bitterness (acrid flavour) in potatoes is solanine or its component solanidine;

(2) Solanine is present in greatest abundance in irradiated sprouts, flowers, leaves and skin, in that order;

(3) Storage conditions do not affect solanine content so much as duration of tubers in storage;

(4) Immature and small tubers are more likely to be high in solanine than are mature tubers; and

(5) Varietal differences may be pronounced, as also may be differences between tubers of one variety.

ANALYTICAL PROCEDURE

For the most part, analyses for solanine and solanidine from potato tubers or other plant parts have employed gravimetric methods wherein the extraction has been made with ether or hot alcohols. Since the substance hydrolyzes readily, the use of mineral acids in analysis is to be avoided, although strong sulphuric and nitric acids are used for certain colour reaction tests. The gravimetric procedures call for extreme care in filtering and in purification by re-precipitation, and rather widely different results by variations of procedure would suggest that they are none too reliable.

In 1937 a report by Pfankuch (21) described a photometric method for solanine determination, and this was modified by Rooke *et al.* as reported in 1943 (22). In the latter report, a hydrolytic method of solanine determination also is outlined, which complements to some degree the detailed and extensive report by Conner (5) in 1937, in which the latter has devised a method of analysis that also employs hydrolysis.

EXPERIMENTAL MATERIAL—PROCEDURE AND RESULTS

The trials that were undertaken during October to March of the 1949-50 winter were essentially of a preliminary nature, and no attempts were made to determine actual solanine content in tubers. Twenty 10-lb. samples of Netted Gem tubers were collected from four producing areas in Alberta, and these were cooked at 15-lb. pressure for 10-15 minutes, without peeling. They were rated as "bitter" or "not bitter" by a panel of tasters, none of whom had the least difficulty in detecting the typical acrid and lasting taste when it was present. Three of the districts (Peers-Edson, Lethbridge and Brooks, with six samples from each) showed three "bitter" and three "not bitter" samples from each, while the fourth district (Edmonton, two samples) showed no bitterness.

Three "bitter" and three "not bitter" samples were chosen for further testing and 30-lb. samples of tubers were secured from each source. These were each divided into six 5-lb. lots and subjected to storage under the following conditions:

Storage treatment	Temperature range (° C.)	Light conditions
1	0 – 5	Darkness
2	5 – 10	Darkness
3	5 – 10	Light*
4	10 – 15	Darkness
5	10 – 15	Light*
6	15 – 20	Darkness

* Subdued winter daylight through basement windows.

Three cooking tests were made, on January 7, February 11 and March 17. Like those of the initial tests, the tubers were washed but not peeled, and were pressure-cooked until the flesh was cooked through. The results were expressed numerically by means of the following rating system, wherein the presence and degree of bitterness was noted in the cortical layer of cooked tuber flesh:

0 = Not discernible
1 = Trace
2 = Slight
3 = Moderate
4 = Marked
5 = Very marked

The complete table, containing readings from 108 tests (6 treatments, 6 samples and 3 times), is found as an appendix to this paper. No attempt was made to apply statistical analysis to the data, but they are summarized in the following tables.

TABLE 1.—EFFECT OF TESTING DATE ON TUBER BITTERNESS IN NETTED GEM. MEAN BITTERNESS RATINGS FROM 0 TO 5 ("NIL" TO "VERY MARKED"). EACH RATING MEAN IS CALCULATED FROM 18 READINGS

Date of cooking test	Three samples—originally not bitter*	Three samples—originally bitter*	Mean
Jan. 7, 1950	2.5	2.3	2.40
Feb. 11, 1950	2.8	3.0	2.90
Mar. 17, 1950	1.9	1.6	1.75
Mean	2.40	2.30	2.35

* Selected from October cooking tests on a larger number of samples.

TABLE 2.—EFFECT OF TEMPERATURE ON TUBER BITTERNESS IN NETTED GEM. MEAN BITTERNESS RATINGS FROM 0 TO 5 ("NIL" TO "VERY MARKED"). (NUMBER OF READINGS IN PARENTHESES)

Storage temperature—° C.	Three samples—originally not bitter	Three samples—originally bitter*	Mean
0 – 5	2.3 (9)	2.9 (9)	2.60
5 – 10	3.0 (18)	2.5 (18)	2.75
10 – 15	2.1 (18)	2.2 (18)	2.15
15 – 20	1.9 (9)	1.7 (9)	1.80

* See footnote, Table 1.

TABLE 3.—EFFECT OF SUBDUED LIGHT ON TUBER BITTERNESS IN NETTED GEM. MEAN BITTERNESS RATINGS FROM 0 TO 5 ("NIL" TO "VERY MARKED"). (NUMBER OF READINGS IN PARENTHESES)

Light exposure	Three samples—originally not bitter*	Three samples—originally bitter*	Mean
Nil	2.2 (36)	2.3 (36)	2.25
Weak and indirect daylight	2.7 (18)	2.3 (18)	2.50

* See footnote, Table 1.

Records also were taken on the degree of bitterness present in the medullary or pith tissue of the tuber, but only in 18 cases of the 108 tests made could traces of bitterness be noted, and these samples were scattered quite casually through the test material, with no one storage period, temperature or exposure to light affecting the tubers more noticeably than another.

The degree of sprouting was noted, and followed a pattern that could be anticipated; that is, by January 7, maximum sprout length was less than one inch even when stored at 15-20° C., but by March 17, potatoes

in the warmest storage, in darkness, had sprouts up to 18 inches long, and even those in the 5-10° C. storage in darkness had sprouts to 13 inches. Regardless of temperature, sprouts remained less than one inch long by March 17 when the tubers were exposed to daylight. The length of sprouting did not appear to show any correlation with the presence or incidence of bitterness in the potato tubers.

Apart from the usual drawbacks that pertain to the "taste" method of obtaining data, it was clear that the bitterness found at ratings = 4 and 5 was sufficient to retard the tests considerably. Even when only one tuber from each sample was tasted, there were 36 tubers to be rated at each cooking date, and quite often it would be necessary to wait for 15 to 30 minutes after sampling a very acrid-tasting tuber before continuing with the tasting. Sometimes, where several bitter tubers were encountered, a burning sensation in the region of the pharynx would become so pronounced and uncomfortable that further judging was delayed for several hours.

One drawback to these preliminary trials has been that they were initiated too late in the season of 1949 for reliable and detailed recording of the pre-harvest and immediately post-harvest conditions. One of the samples that was stored for winter testing was grown at the University of Alberta, and the plants were hilled somewhat more than is general practice throughout Alberta. They were dug in late September and pitted in the field until the common storage room had reached about 5° C. This sample proved to be by far the least affected with bitterness, and on 8 only of the 18 tests was a trace (ratings of 1 or 2) of acrid flavour noted.

It was noted that when the cortical flesh of a tuber tasted markedly bitter, this layer of flesh often was distinctly yellowish in colour, which appears to confirm Mayfield's observations (15) concerning bitterness in the same variety.

DISCUSSION OF RESULTS

Observations of definite trends are not easily made from the results shown in Tables 1 to 3. In Table 1, where the three dates of cooking are compared, there is an upward trend in bitterness amount between January 7 and February 11, then a fairly marked downward tendency by March 17. This is noted both on those samples that were bitter when tested in October and on those that were not bitter, though there is essentially no difference in the latter two categories by the time they had been stored for three months.

A peculiar result that appears in all tables is that the three samples that showed no bitterness in October average higher in acrid taste in the winter months than those that originally showed marked bitterness. During the progress of this trial there has been no occurrence that would offer a reason for this result; nor does a search of the literature suggest that this particular phenomenon has been observed previously.

In Table 2 the four storage temperatures are compared. As with the times of testing (Table 1) there are differences to be noted here. The trend is that the warmer the storage, the less bitterness is accumulated or maintained. Temperatures above 10° C. (50° F.) were obviously associated here with less bitterness, but of course allowed more rapid withering and

senescence onset than lower storage temperatures. The numbers of samples tested would seem to be adequate to establish this as a fairly definite trend, even although the testing technique could not be considered very accurate.

Exposure to subdued light appears to have prevented development of bitterness to a limited extent in those samples that started their storage-life free of the disorder, but has not affected tubers that were bitter to begin with. This would seem to bear out the findings of Wolf and Duggar (23) who reported in 1940 that, whereas in growing shoots with adequate carbohydrate supply solanine was synthesized independently of light, nevertheless, the photoperiod influenced both distribution and synthesis in young (10-week-old) plants. The potatoes that already were bitter at the beginning of their storage-life may conceivably have reached a somewhat more advanced stage of maturity than the others, and were therefore not so subject to light influences during the January to March storage period. On the other hand, the fluctuations in bitterness of the cortex during the three months mentioned are not so easily explained on this basis, unless the immaturity of the "originally not bitter" potatoes was reflected in a less adequate supply of carbohydrates and this in turn was a factor contributing both to rapid synthesis in early storage and to more stability in solanine content after it was synthesized.

As mentioned earlier, there is general agreement among investigators that immature potatoes are found to have a higher solanine content than mature ones, although the intimation in most cases is that field-maturing is meant, rather than any maturing that occurs after harvest. While this does nothing to clarify the present picture in so far as Alberta Netted Gems are concerned, it should be pointed out that the growing season during 1949 was such that only careful producers under irrigated conditions were able to harvest field-matured tubers. At the time of the October (initial) readings on 20 tuber samples, twelve from irrigated districts were secured, and of these, six proved to be bitter. It was possible to obtain only one "bitter" and one "not bitter" sample from these districts for the winter tests, but these did not appear to react differently from similar lots that were grown under non-irrigated culture.

While there has been no proof, during the trial described here, that the "bitterness" was due to solanine content, and although Mayfield (15) finds no association between solanine content and acrid flavour, the bitterness observed in Alberta potatoes from the 1949 crop corresponded so exactly to the descriptions recorded by other workers (3, 6, 7, 8, 12, 17) that there is little doubt in the author's mind that the two are related in this instance.

CONCLUSIONS AND DISCUSSION OF FUTURE PROJECTS

It is obvious that the scope of the work done to date is far too limited to answer many of the questions that may be raised, by both producers and research workers. It appears to be equally obvious that much of the previous research has resulted in contradictory conclusions that cannot be relied upon to provide the information needed by Western Canadian potato producers, so that the likelihood of bitterness in the produce may be reduced or entirely eliminated. The susceptibility of the Netted Gem

variety to bitterness, whether or not high solanine and solanidine content is the cause, seems to be established from reviews of published reports, from discussions with growers and from experience gained in the present trial. Of 19 potato varieties grown in the University of Alberta variety trials in 1949, traces of bitterness were observed in only one variety besides Netted Gem.

The trouble cannot be overcome merely by stating that Netted Gem should be replaced by another variety, because at the present time, and due to an amalgam of widely differing factors, there is no other variety known that so well suits the consuming public over a very large area in Canada and the western United States. It thus appears that information regarding casual factors is a prime prerequisite to control or alleviation. And in order to study these factors thoroughly, a reliable method of solanine analysis must be decided upon. This will necessitate an intensive study of all those used in the past, as well as the possible adaptation of improved laboratory equipment to the development of new analytical procedures.

Thus two main problems are highlighted at the conclusion of the preliminary work:

(1) A comparison of methods of analysis for solanine in potato tubers; and

(2) An application of the most reliable method to the determination of solanine in tubers that have been subjected to sundry cultural, harvesting and storage practices; and the actual identification of the tuber component that is responsible for the acrid flavour described herein as "bitterness".

The answers to both of these problems are being sought in two research projects being undertaken by graduate students of the Plant Science Department, University of Alberta. Both projects are being sponsored by and are receiving valuable financial assistance from the National Research Council of Canada. Results obtained will be reported in some detail as they are established.

SUMMARY

The apparent susceptibility of the Netted Gem potato variety to tuber bitterness has led to concern on the part of producers. This in turn has resulted in the initiation of tests at the University of Alberta to determine the distribution of the disorder, and the effects of exposure to light, duration of storage and temperature of storage on the severity of tuber bitterness.

From an extensive review of literature, it was assumed that a glycosidal alkaloid "solanine" is responsible for the bitterness, but no solanine analyses were made in the presently reported trial. The occurrence of bitterness and the degree to which it was present were determined by tasting the pressure-cooked, unpeeled tubers.

Bitterness was found to be concentrated (*a*) in the cortical region of the tuber generally, and (*b*) near eyes particularly. Because of this fact, and because its oral reaction is so exactly like that attributed to solanine, the theory that the bitterness described here is due to high solanine content seems tenable.

Tests made at 5-week intervals during January, February and March show that bitterness increased from early January to mid-February and decreased thereafter until March 17. In general, low temperature storage maintained or caused more bitterness than storage temperatures at above 10° C. (50° F.). Exposure to reduced daylight during short winter days initiated bitterness where tubers showed none in October, but did not affect it where tubers already were markedly bitter in the autumn.

The possible effects of tuber maturity and the photoperiod during storage upon the inconsistent results obtained, are discussed; as also are plans for projects dealing with studies of solanine analytical methods and factors affecting bitterness and solanine content.

ACKNOWLEDGMENTS

The author wishes to acknowledge with thanks the major part played by A. Hutchinson in carrying out the preliminary trials. Thanks also are due to J. W. Marritt, W. Lobay, and P. D. R. McCalla, and the Alberta Potato Growers Committee, for suggestions concerning treatments employed. A. G. McCalla and W. D. Evans have kindly reviewed the manuscript.

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APPENDIX—TABLE 4

RELATIVE BITTERNESS IN ALBERTA NETTED GEM POTATOES (CORTEX ONLY). INDICES RANGE FROM 0 TO 5, REPRESENTING "NIL" TO "VERY MARKED" BITTERNESS

Storage treatment		Sample number*					
		1	2	3	4	5	6
0 – 5° C. Dark	Jan. 7	3	4	0	2	1	4
	Feb. 11	5	4	1	3	5	3
	Mar. 17	2	2	0	4	4	0
5 – 10° C. Dark	Jan. 7	4	4	2	4	2	3
	Feb. 11	4	3	2	5	4	4
	Mar. 17	3	3	0	2	1	0
5 – 10° C. Light	Jan. 7	4	4	2	3	1	2
	Feb. 11	5	4	2	3	3	4
	Mar. 17	5	3	0	4	0	0
10 – 15° C. Dark	Jan. 7	2	3	1	3	0	2
	Feb. 11	3	4	0	3	3	2
	Mar. 17	4	0	0	5	0	0
10 – 15° C. Light	Jan. 7	2	3	2	4	2	2
	Feb. 11	3	2	0	3	1	2
	Mar. 17	4	4	0	4	2	1
15 – 20° C. Dark	Jan. 7	2	3	0	3	0	4
	Feb. 11	3	3	2	2	1	3
	Mar. 17	4	0	0	1	0	1

* Samples 1, 2 and 3 were not bitter in October; 4, 5 and 6 showed distinct bitterness at that time.

LIGHT- AND TEMPERATURE-SENSITIVENESS IN GERMINATING SEED OF TIMOTHY (*PHLEUM PRATENSE* L.).

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INTRODUCTION

The seeds of *Phleum pratense* L. have presented some difficulties in the germination test and it is common knowledge that they are often sensitive to light and temperature. Freshly harvested seed does not respond to the regulation method of seed testing, and better results have been obtained by other methods on such seed.

The purpose of the work reported here is to investigate the drift of light- and temperature-sensitiveness of timothy seed with time.

LITERATURE

In unpublished work done in the Ottawa Seed Laboratory (1) in 1938, a number of samples were tested to compare the effect of prechilling the tests at 6.5° C. for 5 days before completing the test by standard procedure, with tests run by the standard procedure without prechilling. The prechilled lots averaged from 92 per cent to 100 per cent germination while the tests without prechill averaged from 4 per cent to 35 per cent.

In the same year some work was done on timothy at Ottawa by Leggatt (2) in the Seed Research Laboratory. His results showed that darkness was more favourable at high temperature and light at low temperature. Alternation of temperature generally gave higher results than constant temperatures.

Maier (4) pointed out that constant temperatures of 30°, 20° and 12° C. are progressively less favourable for germination, alternating temperatures most favourable, and that light, both with constant and alternating temperatures, exerts a strongly favourable influence. She also claimed that seeds which fail to germinate become "dark hard" after being kept in the dark on the germination-bed; that is, light no longer benefited them. Toole (5) reported that alternating temperatures were better than constant temperatures and that germination was improved by exposure to light.

Some of Leggatt's (3) results with Agrostis agree with those reported for timothy in this study.

He groups the tests in any one sample in 4 classes (A), (B), (C) and (D), depending on the germination response. Timothy samples show similar responses and these are discussed in the body of the paper.

MATERIAL AND METHODS

A lot of timothy of Boone strain harvested on August 1, 1940, from individual plants, and a second lot which had given interesting results in the Seed Laboratory, were used in this investigation. Before threshing the individual heads of the first, they were separated into ripe and green lots which are designated (A) and (B), respectively. The second sample is designated (C).

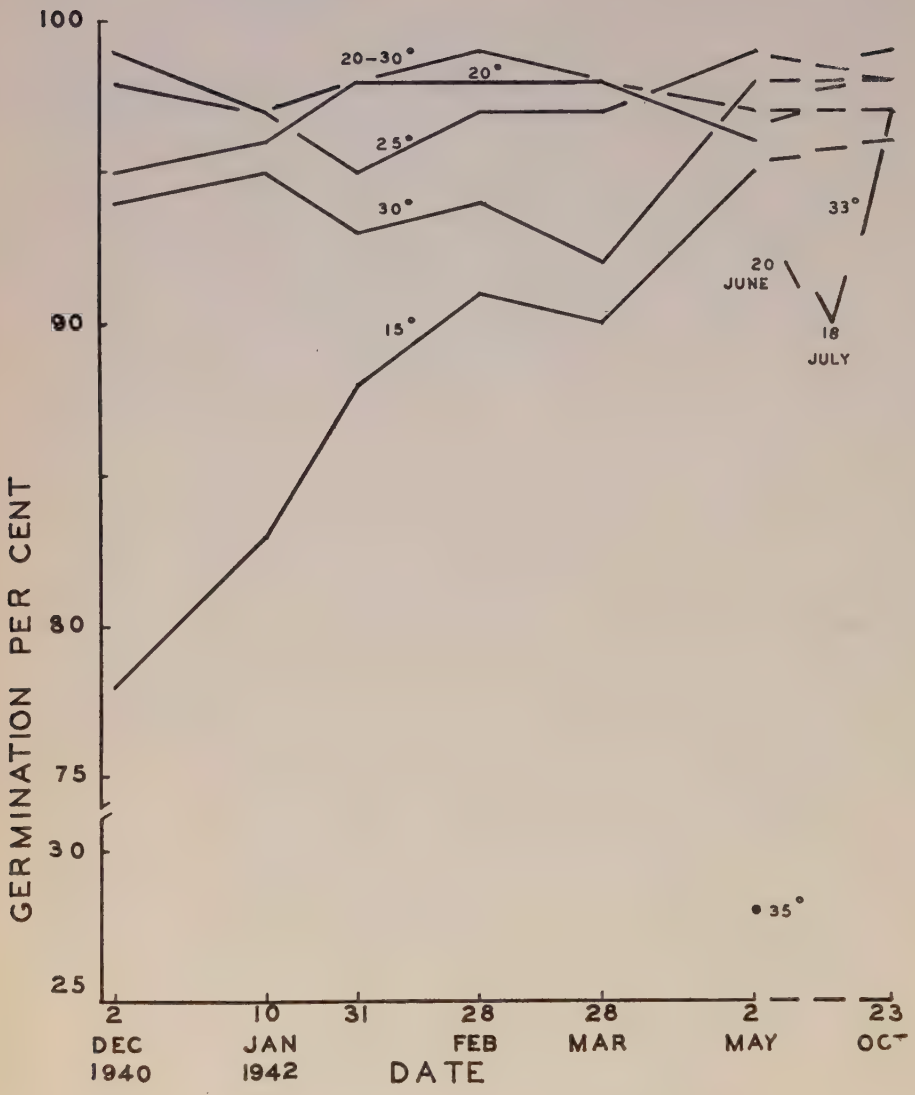

FIGURE 1. Sample A: 10-day counts with light experience.

All tests were carried out using standard laboratory procedure, namely, on top of sand in covered Petri dishes. Four replicates of 100 seeds each were used with two exceptions—2 replicates were used for the 20° – 30° C. alternation throughout, and 3 replicates were used from May until December, inclusive, due to a shortage of seed supply. Where alternating temperatures were used, the low temperature experience lasted $16\frac{1}{2}$ hours and the high temperature $7\frac{1}{2}$ hours. "Dark tests" were placed in film tins which were not opened until this was necessary for the germination count on the 7th day.

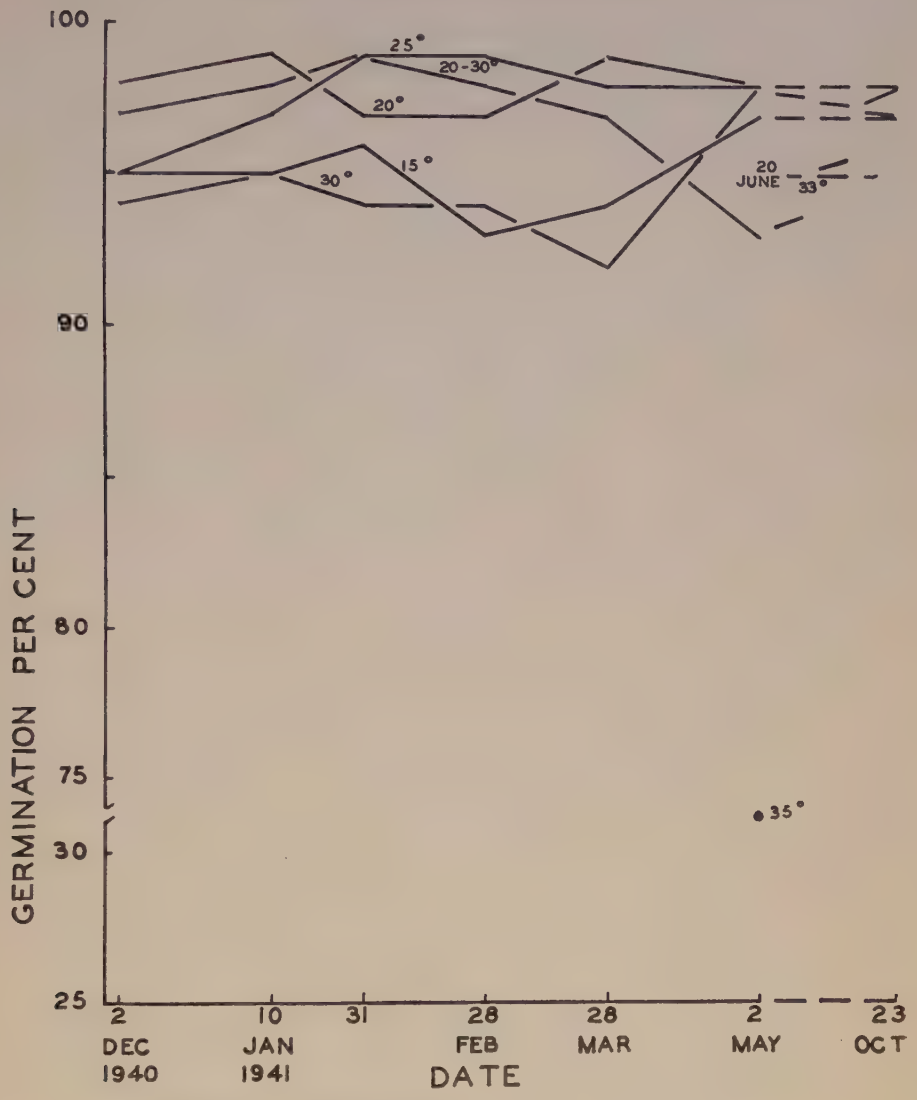

FIGURE 2. Sample B: 10-day counts with light experience.

Temperature control was reasonably satisfactory at all times, but tests could not be carried on during the summer months as the germination tanks were water cooled and could not be kept at the required temperatures. After the first planting, a temperature alternation of 20° to 30° C. in light, as described above, was used as the standard condition and constant temperatures were used for all other tests.

Although in many cases individual differences in germination are not statistically significant, where such differences persist with time a degree of significance must be attached to them. At the 5 per cent level of signi-

ficance, the following tabulation indicates the limits of difference that may be expected in individual comparisons:

Number of seeds used in higher test Number of seeds used in lower test	200 200	200 400	400 200	400 400
Per cent germination in higher test	Differences greater than those given below are significant at the 5 per cent level			
100	2	2	1	1
95	5	5	4	3
90	7	6	5	5
80	8	7	7	6
70	9	8	8	6
60	10	8	8	7
50	10	8	8	7

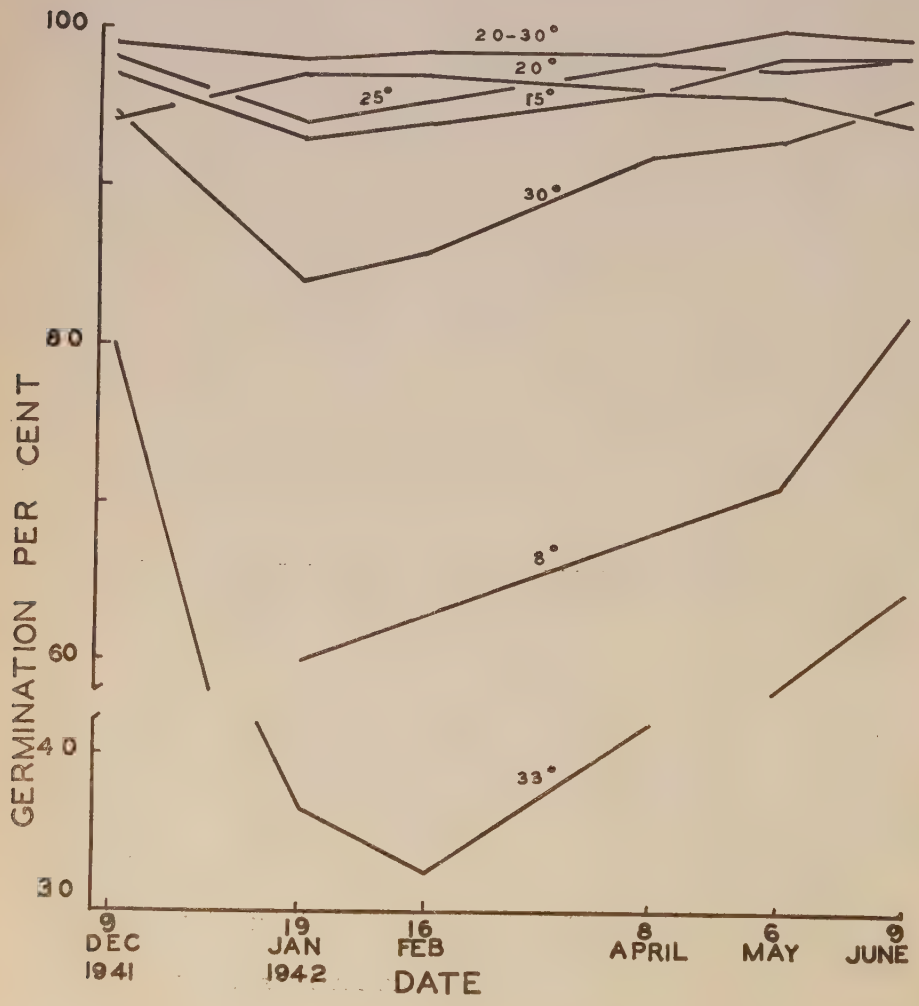

FIGURE 3. Sample C: 10-day counts with light experience.

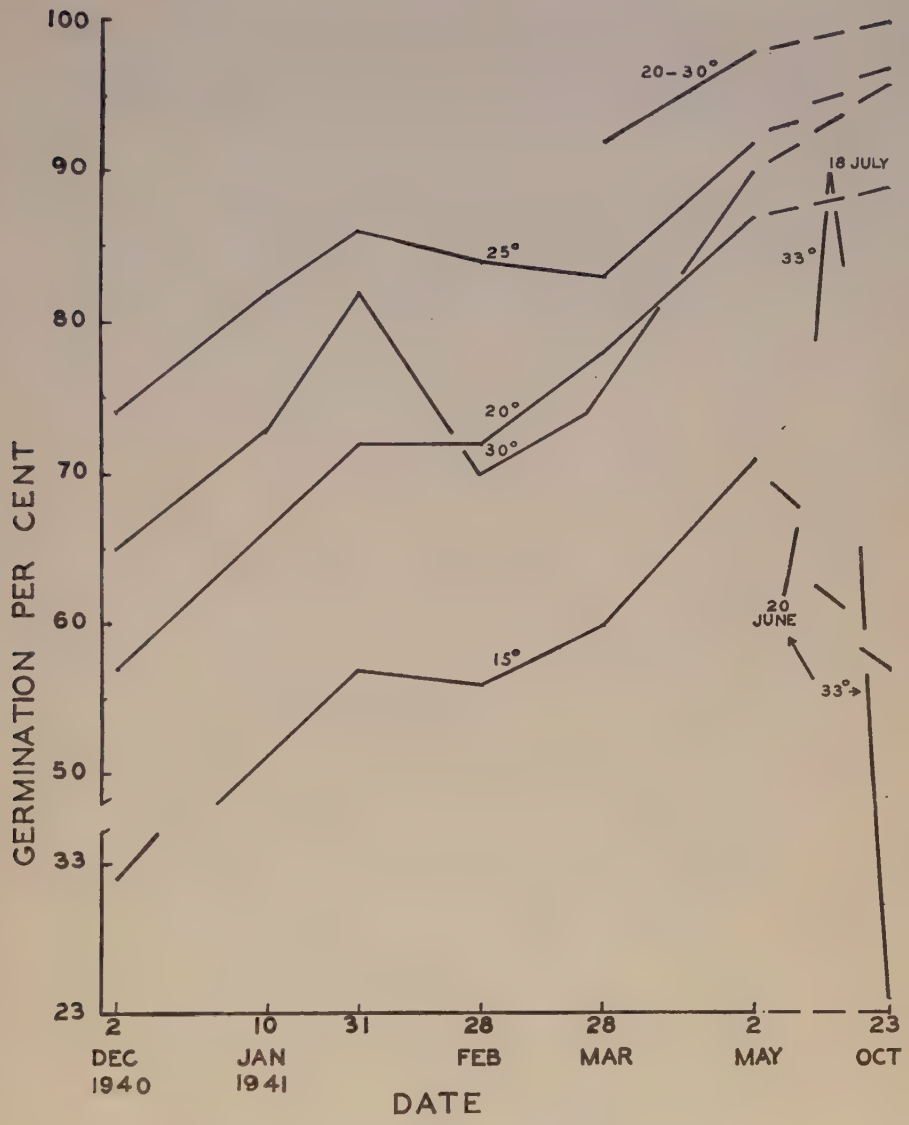

FIGURE 4. Sample A: 10-day counts with dark experience.

PRELIMINARY TESTS

Four sets of alternating temperatures were used on seeds planted on November 8, 1940, as follows:

(*a*) 10° to 20° C. with light and dark experience.
(*b*) 15° to 25° C. with light and dark experience.
(*c*) 20° to 30° C. with light and dark experience.
(*d*) 20° to 35° C. with light and dark experience.

RESULTS

Tests in Light

These were counted on the 5th, 7th, 10th and 12th days. Samples A and B averaged 97 per cent to 98 per cent total germination under all temperatures.

Tests in Dark

These were not counted until the 10th day and were removed on the 12th day. If there were many fresh ungerminated seeds they were transferred to 20° to 30° C. with light for one week. In Sample A, the germination varied from 66 per cent to 88 per cent, and in Sample B, from 69 per

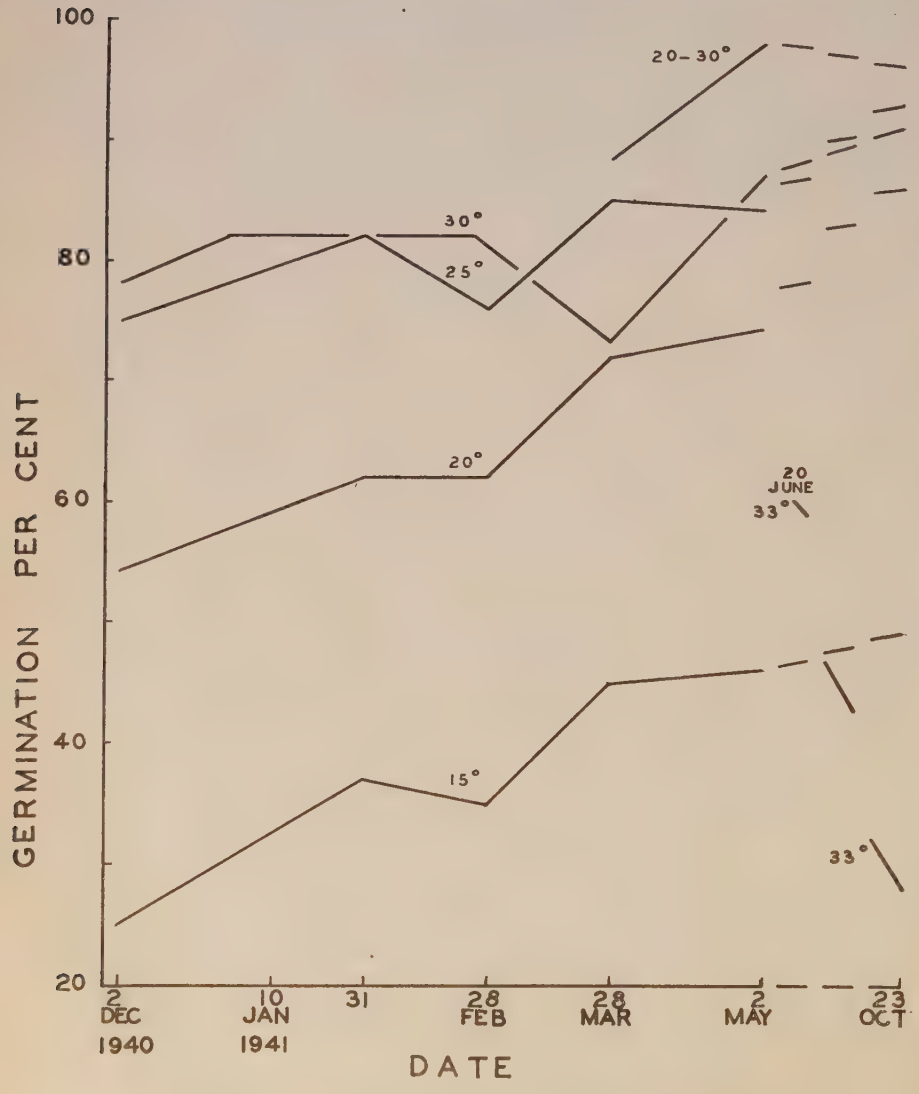

FIGURE 5. Sample B: 10-day counts with dark experience.

cent to 95 per cent, the germination increasing directly with the temperature in both cases. On transferring to light, the samples which contained the most fresh seeds, that is those which had experienced the lower temperatures, germinated from 3 per cent to 17 per cent more.

MAIN INVESTIGATION

The results of the preliminary series showed such a uniform germination, particularly in the light, that the conditions were changed to see if

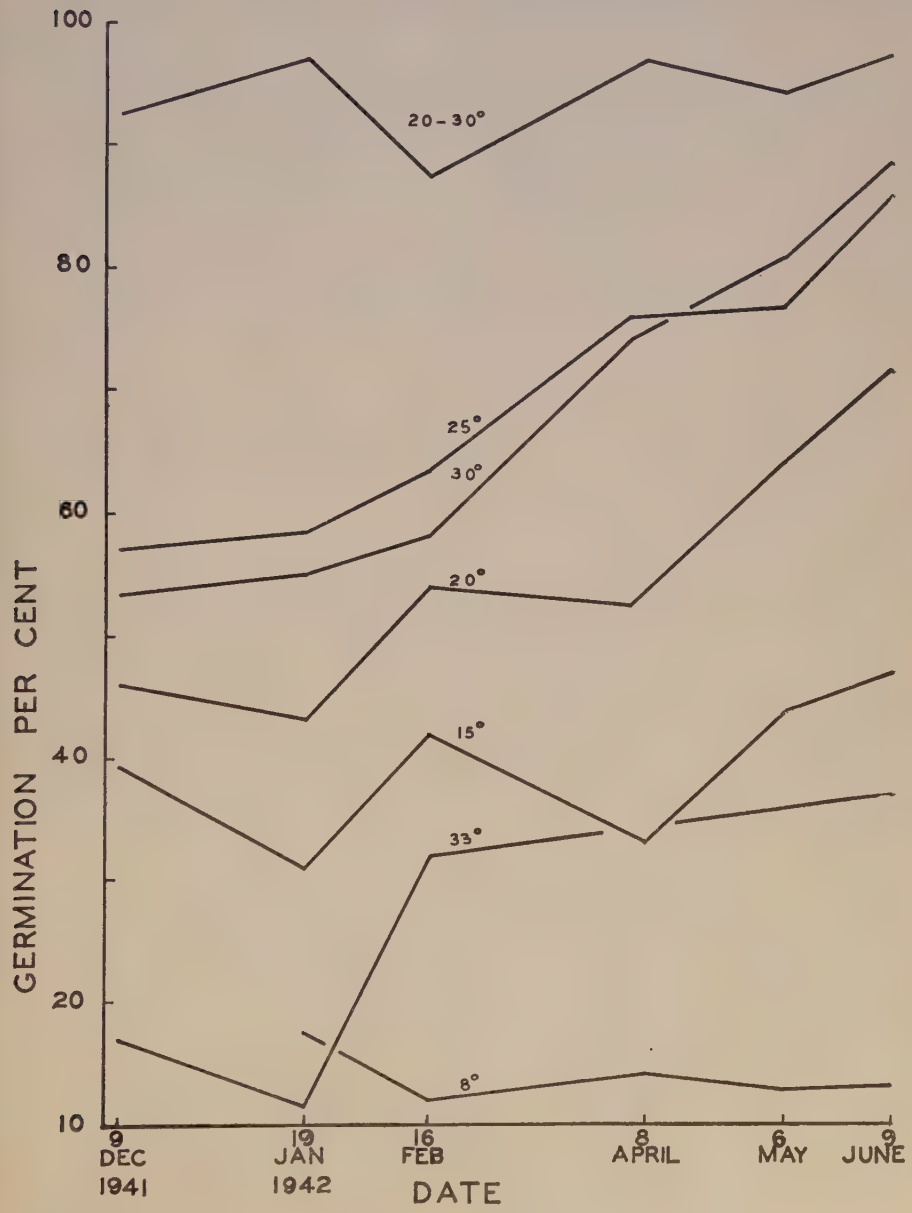

FIGURE 6. Sample C: 10-day counts with dark experience.

something more significant would emerge, so for the remaining tests carried on from December 1940 until June 1942, the following sets of conditions were used:

(*c*) 20° to 30° C. with light and dark experience (standard conditions)
(*e*) 15° C. with light and dark experience
(*f*) 20° C. with light and dark experience
(*g*) 25° C. with light and dark experience
(*h*) 30° C. with light and dark experience
(*i*) 35° C. with light and dark experience (only one test)
(*j*) 33° C. with light and dark experience } (these were not used in all the tests)
(*k*) 8° C. with light and dark experience }

Unfortunately, it was not appreciated at the time that it was advisable to carry out the 20° to 30° C. tests in the dark as well, and, as seeds were limited, this test was omitted for part of the plantings. From December 1940 until Samples A and B were last tested in October 1941, eight plantings were completed. Since at that time the sensitiveness to conditions had passed off, a new sample C was chosen.

Sample C was used from December 1941 to June 1942. Counts were made at 7 and 10 days, followed by removal to 20° to 30° C. temperature alternation with light and a final count at 14 days.

RESULTS

The 10-day counts are presented in graphs 1 to 9. Graphs 1 – 3 give the results on Samples A, B and C tested at various temperatures in light; graphs 4 – 6 give the same for tests in the dark; graphs 7 – 9 give the light/dark ratios at different temperatures.

Effect of Temperature in Light

In Samples A and B, temperature has a negligible effect at the time of the last test. The mature Sample, A, germinated less than the immature Sample, B, early in the season at the lowest temperature, while both samples were adversely affected (May) at the extreme temperature of 35° C. but not at 33° C. This remarkable sensitiveness in the region of 35° C. is of noteworthy interest. Sample C was affected to a much greater extent by temperature and at most temperatures an initial fall in germination was succeeded by a rise.

Effect of Temperature in Dark

In all tests absence of light produced a marked depressive effect on germination early in the season, except with Sample C at 20° – 30° C. alternation. This effect decreases with time except in Samples A and B where the October planting at 33° C. shows a sharp decline in germination. Alternation of 20° – 30° C. was most favourable throughout. Constant temperatures in general gave a progressive increase in germination with time, the optimum being between 25° and 30° C.

Figures 1 to 6 show in many of the graphs a drop in percentage germination within the period February to March. This dip seems to occur too regularly to be accidental and is probably due to partial secondary dormancy setting in at about this time.

Effect of Temperatures on Light/Dark Ratio

The graphs of the light/dark ratio are about mirror images of the percentage germination graphs. The ratios show a tendency to fall with increasing temperatures followed by a rise as the highest temperatures are reached and, with one or two exceptions, there is a general tendency for the ratios after a sharp initial drop followed by a rise, to fall with time. In all samples the 20° – 30° C. ratio is practically a straight line showing that the effect of light is negligible. Constant temperatures of 8° C., 15° C.,

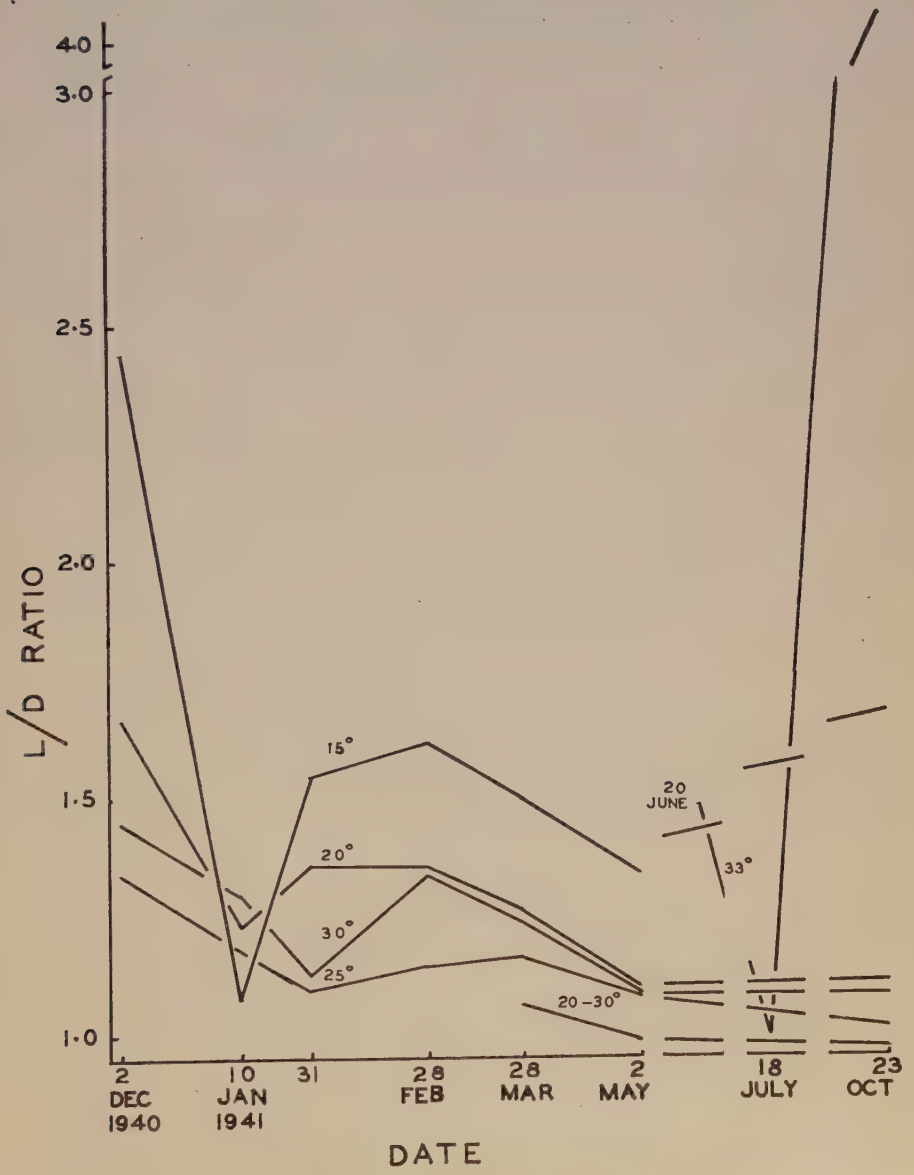

FIGURE 7. Sample A: Light/dark ratio of germination.

and 33° C. give comparatively high values showing positive light-sensitiveness at these temperatures. It is interesting to note that Sample C is negatively light-sensitive at 33° C. during the one reading in April.

SPEED OF GERMINATION

Speed of germination is represented by the ratio:

$$\frac{\text{7-day count}}{\text{10-day count}} \times 100 .$$

For the 15° C. tests, this is shown in Table 1. Under the other conditions 90 per cent to 100 per cent of the germination took place in the first 7 days.

TABLE 1.—SPEED OF GERMINATION; TESTS IN LIGHT AND DARK; AT 15° C.; 7-DAY COUNT AS PERCENTAGE OF FINAL COUNT

Sample	Date	Dec. 2	Jan. 10	Jan. 31	Feb. 28	Mar. 28	May 2	June 20	Oct. 23
A	In light	68	71	61	76	79	90	—	98
	In dark	90	70	93	86	97	97	—	98
B	In light	85	83	82	80	89	99	—	100
	In dark	92	48	87	77	77	89	—	94
Sample	Date	Dec. 9	Jan. 17		Feb. 16	April 8	May 6	June 9	
C	In light	96	63	—	93	—	99	99	—
	In dark	59	87	—	93	—	97	93	—

Note.—At the other temperatures used, the speed of germination was uniformly high, varying from 90 per cent to 100 per cent.

The tests at 15° C. showed markedly lower speed of germination up to about March with the exception of Sample C which showed low germination speed in only one month, January, when tested in light and December when tested in the dark. Samples A and B when tested in the dark both showed high speed of germination in December, which, however, fell to a low figure in January.

DISCUSSION

The timothy seed used in this investigation was definitely light-sensitive, especially early in the season, but given light, it was not temperature-sensitive except at extremes of high and low temperature. Sample C in light, however, was more sensitive to extreme temperatures than either A or B, but this sensitiveness decreased with time.

Alternating temperatures were extremely favourable and for this reason only the 20° to 30° C. alternation was continued and used as a standard throughout.

In the dark the germination first increases with increasing temperature and then declines. This effect is less noticeable in the light but is still

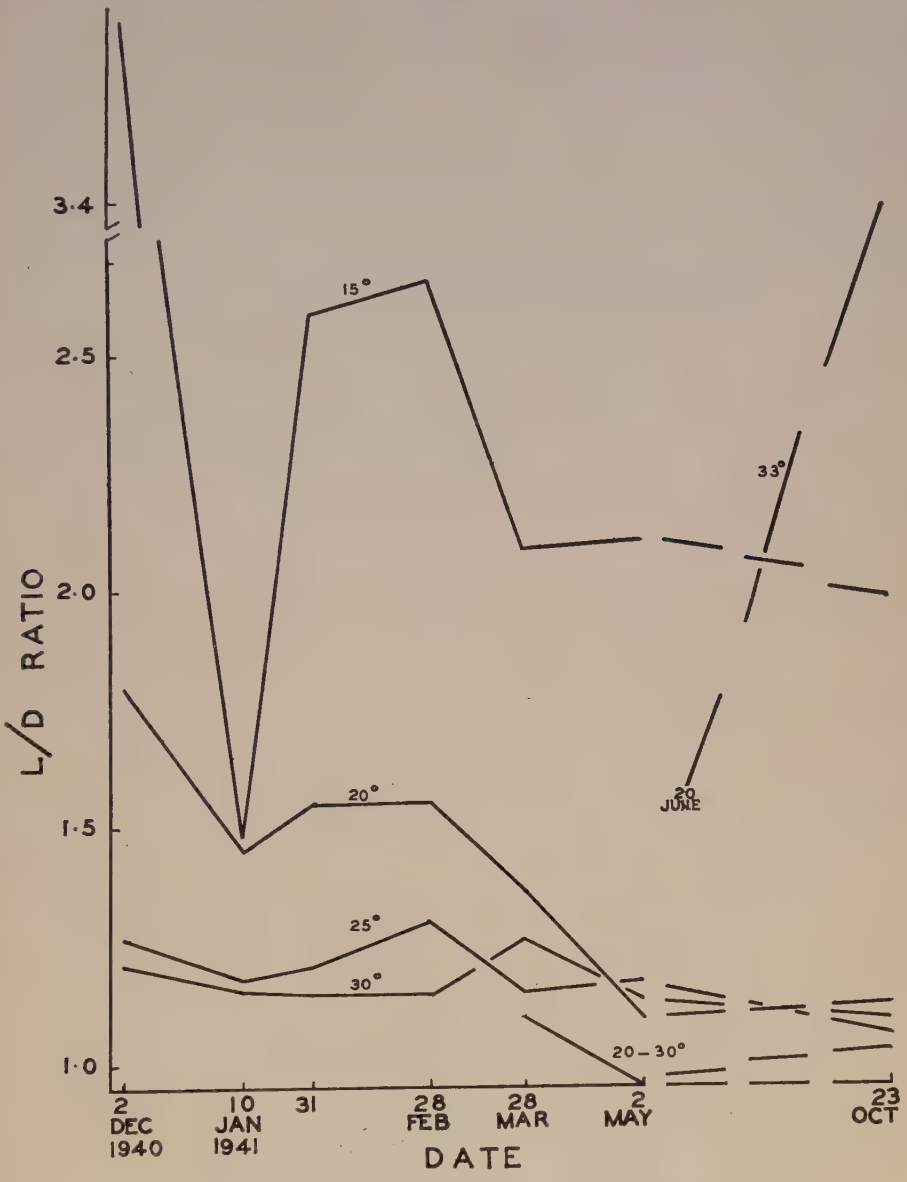

FIGURE 8. Sample B: Light/dark ratio of germination.

marked in the case of Sample C. At the time of the last test there is a constant temperature at which the seed seems to be practically insensitive to light which is referred to later as the optimum constant temperature.

In the light, there was little difference in the response of Samples A (mature) and B (immature) except that in the early tests, B gave complete germination at 15° C. while A did not. These observations are in accord with the general experience of seed analysts with seeds of the grass family, that the germination of immature seed is favoured by low temperature and

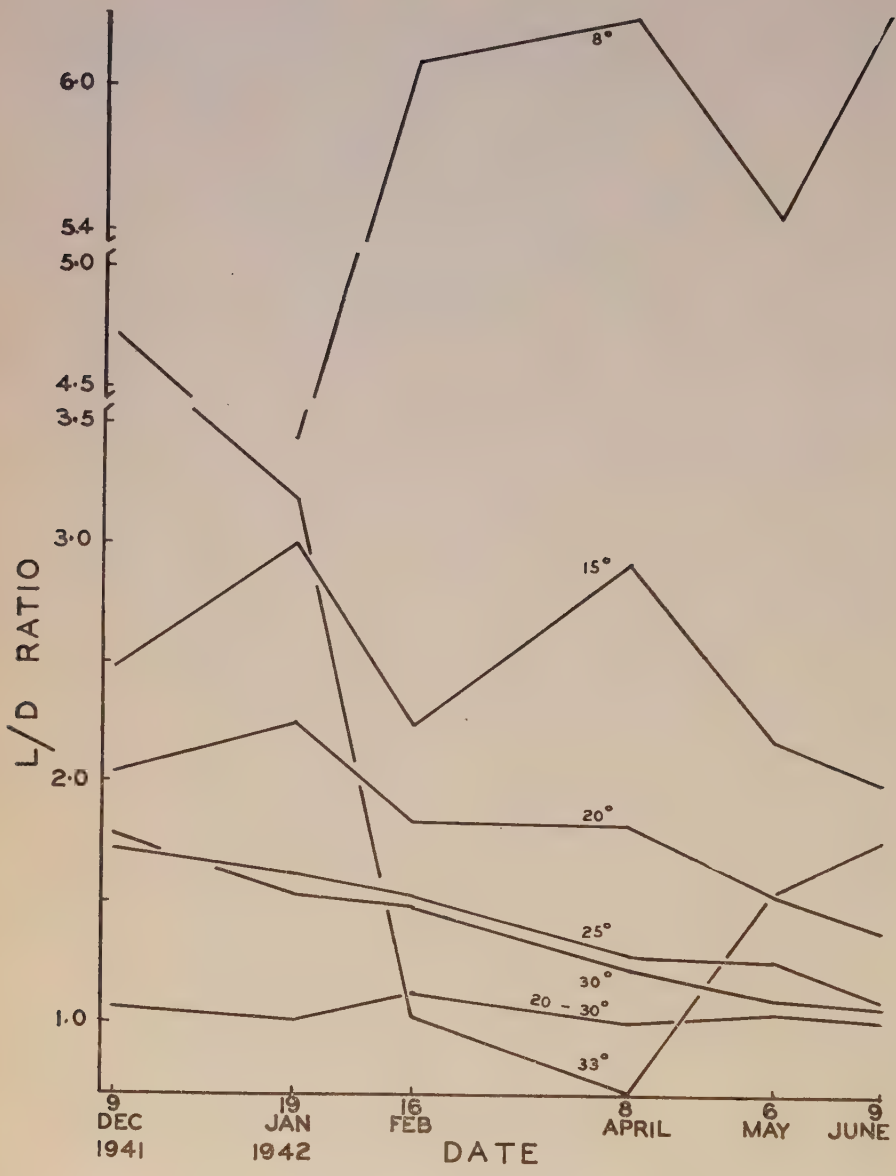

FIGURE 9. Sample C: Light/dark ratio of germination.

light. As Sample B aged, the difference in behaviour between it and Sample A became less. In the dark, however, A gave generally somewhat higher responses to the various temperatures than B, a fact which is clearly shown in the graphs of the light/dark ratio, Figures 7 and 8.

Unfortunately, the antecedents of Sample C were not known, so that its markedly different responses cannot be accounted for in relation to its age or maturity.

Comparison with Other Workers

Leggatt (2) predicts from his light/dark ratio plotted against temperature that at a certain temperature we should find the sample is light-insensitive and suggests that this would be at about 27° C. The three samples worked with here seem to behave similarly, the optimum constant temperature always falling between 25° and 30° C.

In contrast to the results reported by Maier, 30° C. constant temperature was not always more favourable than 20° C. (Sample C in light at 20° C. germinated higher throughout). Again, contrary to Maier's findings, the seeds which failed to germinate in the dark at any temperature, when given light and an alternation of 20° to 30° C. for 4 days, germinated from 50 per cent to 97 per cent.

The results obtained here confirm Toole's (5) conclusion that in general alternating temperatures are better than constant temperatures.

Leggatt (3) in his work on Agrostis refers to what he calls the "February Dip". This is a feature common to all the graphs in his paper and it shows up here in almost every graph in either February or March. It would appear that this partial secondary dormancy is common to more than one genus of the grasses.

It has been pointed out that, in the dark tests, the 7-day count gave results almost equal to the final germination figure (within 95 per cent to 100 per cent except in a few cases). This is similar, in the case of the 20° C. data, to that found by Leggatt (3) for Agrostis and the seeds which germinate promptly at this temperature in complete absence of light may be considered germinating-ripe and referred to Class A (3). The rising curves given by the 20° C. tests in the dark (Figures 4, 5 and 6) show that Class A seeds increase with time, as in Agrostis. The early stages of the curves for 20° to 30° C. in the dark unfortunately were not obtained but, at least in Sample C, the curve does not rise as steeply as the 20° C. curve (Figure 6), a point of difference from the corresponding curves of Agrostis. Nevertheless, the difference between the 20° C. dark curves and the 20° to 30° C. dark curves may be ascribed to Class B seeds, namely, those which may be brought to germinate by temperature alternation in absence of light. As, however, the 20° to 30° C. dark curves maintain a consistently high level, it would appear that Class C seeds (i.e. those requiring both light and temperature alternation) are lacking. Since high germination was obtained with all samples under the most favourable conditions, it appears that Class D seeds (i.e. dead or fully dormant seeds) are present in very small proportion. In the work on Agrostis, it was concluded that the Class B seeds were the ones affected in the occurrence of the "February Dip". This conclusion fits in well with the present data and it is probable that the explanation of the occurrence of this secondary dormancy is similar.

SUMMARY

Germination tests on the seed of *Phleum pratense* L. were begun in November 1940 and carried through until June 1942, with the object of studying the drift with time of sensitiveness to light and temperature.

Seeds of the three samples used proved to be light-sensitive at both low and high temperatures, and at all temperatures early in the season, but there is a constant temperature (between 25° and 30° C.) at which the seed is relatively light-insensitive.

Some degree of secondary dormancy is developed in the period February to March, as shown by lower germination response at some time during this period. Seeds which have failed to germinate in the dark at any given temperature are not thereby rendered dormant, but germinate when placed under favourable conditions. There is a gradual decrease in both temperature- and light-sensitiveness with time.

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